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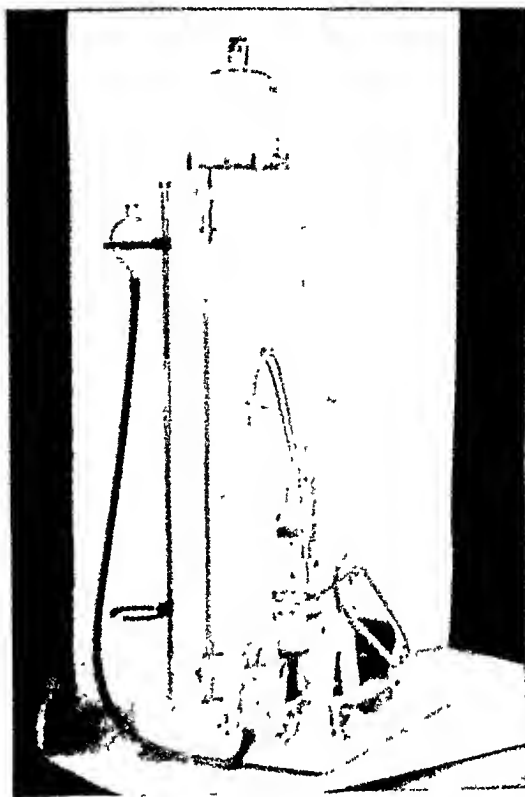
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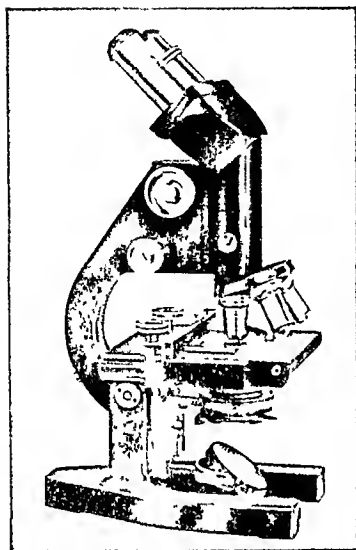
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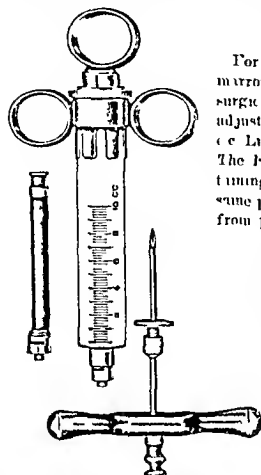
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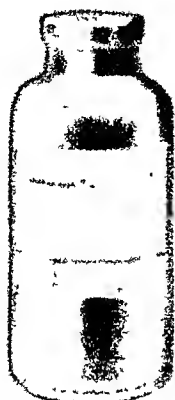
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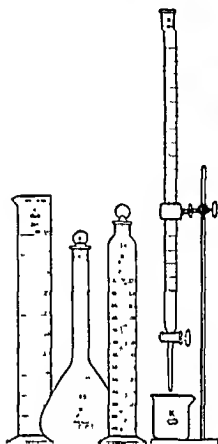
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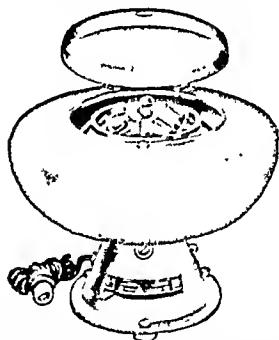
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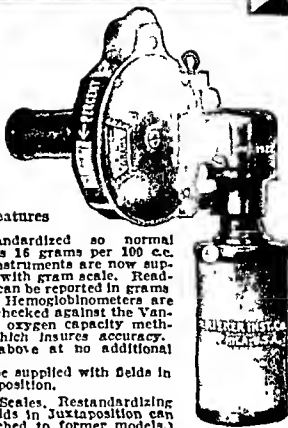
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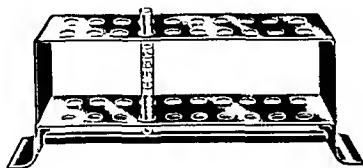
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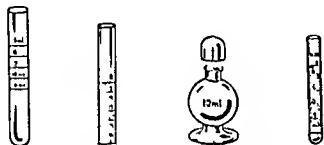
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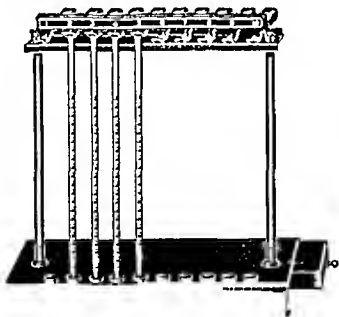
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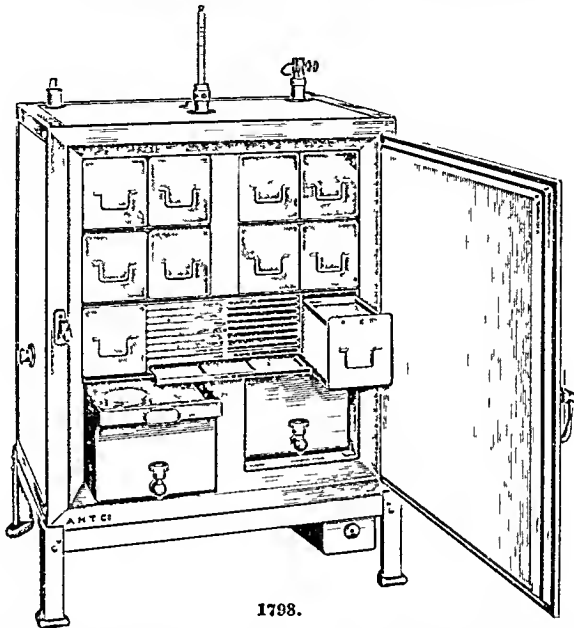
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ADRENAL CORTEX EXTRACT*

A CONSIDERATION OF ITS USE IN VARIOUS TYPES OF ADRENAL INSUFFICIENCY AND RELATED CONDITIONS

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IN COMMON with other endocrine structures, the adrenal glands have attracted considerable interest, particularly in recent years because of the more precise knowledge of the function of the cortex and the availability of potent extracts. In view of the wide scope of investigation in various quarters and the growing literature, it is now possible to chart the trend of such developments. These may be broadly divided into three general groups: (1) The relation of the adrenal cortex to mineral metabolism as noted in the alterations induced by experimental extirpation and the fairly similar changes in Addison's disease, (2) the interrelationship of the adrenals to other endocrine structures and their part in pluriglandular syndromes, (3) certain associated effects and functions not clearly delineated, such as the relation of the cortex to immune processes, vitamin metabolism and similar topics. The first group is so far the most accurately studied though the practical application of this knowledge is restricted to the small group of Addison's disease. The evidence in the latter two groups is still equivocal, the subject matter covers a wide range of clinical problems and unfortunately is a fertile field for vague speculative articles not based upon substantial scientific data. Attracted to these problems in the course of experiments upon the preparation

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of a potent adrenal cortex extract,* we have thought it of interest to epitomize the results of adrenal cortex therapy upon sixty patients who have been considered suitable for inclusion in one or more of the three categories mentioned above. The age periods in this study ranged from infancy to the sixth decade. A few newborn infants who were treated for adrenal hemorrhage syndrome or marasmus are included, though no encouraging results were obtained.

In order to indicate the theoretical basis and limitations of the clinical use of adrenal cortex extract, it is desirable to review briefly the experimental linking of the adrenal cortex to mineral metabolism.

Harrop¹ and his collaborators studied the changes produced in adrenalectomized dogs by the cessation of daily injections of cortical extracts after a maintenance level had first been established. By this method and by the use of nembutal anesthesia, a number of variables were eliminated. The principal changes induced by the cessation of cortical extract were, hemoconcentration with loss of water and salt, retention of blood urea nitrogen, increase of plasma proteins, lessened blood flow and reduced basal oxygen consumption. The loss of fluid was found to be mainly by renal excretion rather than movement into the tissues or gastrointestinal tract. The fall of chlorides was accelerated if salt had been restricted in the diet. It is well established that the movement of the chlorine anion between the blood plasma and cells of the blood and tissue is such that it enables the cell buffers to contribute to the stability of the acid-base equilibrium. The net result of such loss of chlorides with that of sodium and some carbonate is one of compensated alkali deficit. The nitrogen retention which has been found both in experimental and clinical observations has usually been attributed to renal failure consequent upon lack of fluid and fall in blood pressure. However Harrop and others believe that it may be part of a compensatory mechanism to restore osmotic equilibrium after marked loss of electrolytes. Since the approximately 100 m. eq. per liter of chloride ion make up about two-thirds of the ions of the plasma, it is apparent that some readjustment is necessary. Under normal conditions at least, urea is freely permeable through the body membranes and therefore has little value in osmotic equilibrium. Whether this property is reversed in adrenal insufficiency cannot be stated from the evidence so far. Upon resumption of cortical extract injections, they found a restoration of the electrolyte pattern.

Loeb² has reported similar changes in adrenalectomized dogs and has indicated that the loss of sodium from the body is an important factor in the development of insufficiency in this animal. He also believes that this loss is by the kidney and not due to vomiting, diarrhea or loss into the tissues. He considers this sodium loss a primary factor due to failure of some regulatory function of the adrenal cortex rather than secondary to loss of fluid or to combinations with excreted acids as in diabetic acidosis. A rise in serum potassium and nonprotein nitrogen was also noted by Loeb. Substantially similar results have been reported by Zwemer and Sullivan³ in adrenalectomized cats. Numerous clinical observations have indicated that analogous conditions of hemoconcentration and electrolyte dislocations occur in Addison's disease.

*During part of this investigation we used an extract prepared in our own laboratory, later we used a similar extract prepared by the Wilson Laboratories. These extracts are free of adrenalin.

This explanation has not satisfied all observers. Britton and Silvette⁴ are among the protagonists of the theory that a disturbance in carbohydrate metabolism produces the manifestations of adrenal insufficiency. They believe that the adrenalectomized animal suffers from a critical fall in glucose and glycogen with consequent collapse and a shift of fluid from the blood to the liver and muscle tissues. Hypoglycemia though a characteristic finding in experimental adrenal insufficiency and frequently in Addison's disease has been usually considered a secondary factor. The hypoglycemia may be severe enough to be a factor in episodes of collapse in patients with Addison's disease, and we have seen typical hypoglycemic shock during a glucose tolerance test in such a patient. The question as to whether the experimental adrenal insufficiency is a counterpart of the clinical disease can be answered in the affirmative with the exception of the absence of pigmentation in the experimental animal; the blood pressure in the adrenalectomized dog is low only in the late stages after dehydration has set in.

The clarification of the chemical disturbances in adrenal insufficiency has naturally indicated the methods by which these changes may be reversed. It has been repeatedly demonstrated that severe manifestations of adrenal insufficiency in both human beings and animals may be prevented for some time by the ingestion of large amounts of sodium chloride, particularly if reinforced with sodium bicarbonate or sodium citrate even without the necessity for also using cortical extract. It is also true that adequate amounts of a potent adrenal cortex extract will assure a normal electrolyte balance without any special efforts to increase the salt content of the diet. At the Mayo Clinic, Wilder⁵ and collaborators in a careful study of three patients found that critical symptoms may be delayed if the potassium intake of the diet is kept down to 1.6 gm. or less daily even under sodium chloride restriction. Kendall⁶ believes that the hormone has two components, an A fraction responsible for normal muscular activity and a B fraction which enables the combined components to bring the raised blood urea noted in insufficient animals down to normal, presumably by enabling them to retain sodium chloride.

Because of these developments the whole subject has become so well defined that it is now possible to recognize subclinical or mild types of Addison's disease by definite even though slight chemical alterations. This phase in itself has greatly enlarged the clinical outlook at first restricted to the rather small group of fully developed Addison's disease. These individuals go about in a constant state of ill health without episodes severe enough to cause hospitalization and without a clear-cut diagnosis. The following case history is typical of this group:

M. K., a white male aged fifty seven years, had a gastroenterostomy performed at the age of thirty-eight, for duodenal ulcer. His complaints when first seen in 1933 were weakness, inability to gain weight, anorexia, occasional vomiting and pain to the left of the umbilicus. His pain had not been relieved by a modified Sippy diet, alkalis and a belt for visceroptosis. On examination he had marked pigmentation of the lips and buccal mucosa. Blood pressure was 75/50; x ray showed a small heart and lung fields suggesting chronic fibroid tuberculosis though the sputum has always been negative for tubercle bacilli. Blood urea nitrogen was 26 mg. and plasma chlorides were 550 mg. per 100 c.c.; serum sodium

varied from 130 to 134 m. eq. per liter. A striking improvement in nutrition, strength and blood pressure was obtained by weekly dosage of only 2 c.c. of adrenal cortex extract. This amount was, however, inadequate to keep the blood chemistry within normal range during the entire interval between injections. The pain has not recurred despite the use of an unrestricted diet.

Another type of case which we have noted with increasing frequency has exhibited manifestations of nutritional deficiency with secondary signs of adrenal failure. Packard and Wechsler⁷ have called attention to a syndrome characterized by malnutritional edema, polyneuritis and signs of adrenal insufficiency. However hypotension and pigmentation were not necessarily present. There were in all 25 patients in our series who could be placed in a miscellaneous group of subclinical or mild adrenal insufficiency though only approximately half of them exhibited definite improvement on cortical therapy.

SHOCK

There is no unanimity of opinion upon the mechanism of shock and there is no intention here to go into the controversial aspects of this subject. It is, however, fair to agree with Swingle, Harrop and others that the chemical changes found in experimental adrenal extirpation simulate to a varying degree those found in surgical shock. Indeed Swingle, Pfiffner, Vars, Bott and Parkins⁸ have prepared a table of similarities which is quite striking. Among the phenomena which are common to shock and adrenal insufficiency, they list: a decrease in blood volume, blood plasma, rate of blood flow, cardiac output and alkali reserve; an increase in hemococentration, blood viscosity, nitrogenous constituents and sensitivity to hemorrhage, trauma and infections. Some of these resemblances have been pointed out by other observers. Atchley⁹ believes that a distinction should be made between medical or anhydremic shock and surgical shock. He believes that the former is accompanied by a primary loss of blood volume due to dehydration with recovery on replacement of fluid. It is possible that some deaths in diabetic coma are of this nature. Transudation of fluid does not occur into the tissues in this type. Traumatic shock on the contrary may be dependent upon a loss of capillary tone, interference with tissue circulation and a resultant loss of circulating blood volume; if fluid or plasma is lost as it is in some cases with toxic capillary change, it presumably is lost into the tissues. Therefore the mechanism of adrenal insufficiency fits into the category of anhydremic or medical shock. In other respects the resemblance of adrenal insufficiency to a shocklike state is well borne out. The sensitivity of the adrenalectomized animal to hemorrhage, trauma, toxins, drugs, and the like is quite marked.

In the application of experimental findings to the clinical use of cortical extract in shock, several serious objections must be met. The fact that the extract replaces the lost function in the adrenalectomized animal does not necessarily mean that similar results will occur in hemoconcentration due to a different cause and a different mechanism. On the contrary the hormone may act in the presence of a disturbed physicochemical state of the circulation such as has been outlined above, even if this state has not been primarily produced by loss of adrenal function. Practical experiences seem to support

the latter view and this would make it a valuable adjunct in the prevention and treatment of surgical and other forms of shock. Some of these conditions were fulfilled in the following case, though the ultimate outcome was unimpaired.

A white female aged sixty-two years, suffering from biliary cirrhosis adhesions about the gallbladder and ascites was seen twenty-four hours after operation in severe shock. Her blood pressure which was 60/40 eighteen hours after operation had been temporarily raised to 100/50 by intravenous glucose in saline solution though the cell plasma ratio still indicated hemoconcentration. Two hours after an injection of 2 cc of adrenal cortex extract, the blood pressure rose to 140/70 and remained well sustained for another twelve hours. The patient died about forty-eight hours after operation with so called "hepatic toxemia."

In the preparation of patients with thrombopenic purpura for splenectomy we have occasionally observed instances of hemoconcentration and in consequence have used adrenal cortex extract in conjunction with other routine procedures to guard against the possibility of shock. Recent evidence that the susceptibility to shock of individuals with the thymic habitus may be related to adrenal insufficiency, suggests the probable prophylactic value of adrenal cortex extract in such patients. The emphasis placed upon the shock aspects of diabetic coma in the reports by Atchley and also Lande¹⁰ would suggest the use of adrenal cortex extract along with the other recommended measures in these instances. If this is contemplated, it must be remembered that there is an element of danger if hydremia instead of hemoconcentration is present and if the heart and kidneys are not competent to meet the demands of an influx of fluid into the circulation. Two patients with extensive burns were treated with adrenal cortex in conjunction with other standard measures. In one patient, a girl of nine, there appeared to be a favorable effect on dehydration and shock, though she as well as the second patient succumbed after several weeks.

RESPIRATORY METABOLISM

Low basal metabolic rates are quite constantly a feature in well developed cases of Addison's disease. The experimental analysis of this phenomenon has been thoroughly studied by Swingle and Pfiffner¹¹. From the clinical view point it can be said that the effect of cortical hormone upon the metabolism of patients with Addison's disease is not constant. However, Greene, Rowntree, Swingle and Pfiffner¹² found that if the basal metabolic rate of the patient before treatment has been low, the hormone has a tendency to raise it toward normal. This increase is not necessarily commensurate with the increase in weight, strength or vitality of the individual. The analysis of these effects is naturally more involved than in the experimental animal and still more so in pluriglandular syndromes exhibiting dysfunction or failure of the adrenotropic and thyrotropic hormones of the pituitary such as Simmond's cachexia and related disorders. It would appear that the blood cholesterol level would be of aid in estimating the thyroid component in the low metabolic rates of Addison's disease and such pluriglandular syndromes as mentioned above. Occasionally extremely high cholesterol readings have been noted in Addison's disease though Harrop and Weinstein¹³ reported normal

figures in these patients even with basal metabolic rates as low as minus 24 per cent. Two of our patients with low basal metabolism though exhibiting different endocrine manifestations did well on adrenal cortex extract after unfavorable response to thyroid extract.

One of these, M. M., a heavily built man aged forty-seven years, complained of asthenia and fatigability; he was found to have hypotension, small areas of pigmentation on the lower lip, a basal metabolic rate of minus 34 per cent and a blood cholesterol of 294 mg. per 100 c.c. though without objective evidence of myxedema. Thyroid extract by mouth was not followed by any improvement and in fact made him more irritable. On weekly injections of 2 c.c. of adrenal cortex extract over a period of three months, the asthenia was definitely improved and the basal metabolic rate six months later was minus 13 per cent. However the systolic blood pressure had increased by only 10 mm. of mercury.

GONADS

Despite the wide acceptance from a clinical and even from a pathologic viewpoint of a connection between adrenal and gonadal function, there is a surprising lack of uniformity in actual animal experiments bearing upon this question. The various hypotheses proposed to explain these results may be found in a comprehensive review by Britton,¹⁴ also in a recent article by Howard and Grollman.¹⁵ The latter attribute the contradictory reports in the literature to impurities in the extract or to spontaneous variability in the experimental animal. They also believe that if alterations in the sexual functions of adrenalectomized rats occur, they merely represent one phase of a general bodily change.

The clinical observations upon this relationship are found in reports which cite improvement in adrenogenital syndromes following surgery upon hyperplastic or neoplastic adrenal tissue. Broster, Gardiner Hill, and Greenfield¹⁶ have performed unilateral adrenalectomy on three patients each representing a different type of this syndrome: pseudohermaphroditism, virilism and Acharh-Thiers syndrome (diabetes, obesity, and hirsutism in women). The most definite improvement was in the patient presenting virilism of the adrenal type. Walters, Wilder, and Kepler¹⁷ believe that cortical adenoma or hyperplasia of the adrenal cortex may be suspected in female patients if they present a syndrome characterized particularly by obesity of face, trunk, and abdomen, hirsutism, hypertension, hyperglycemia, and amenorrhea. In their report several patients presenting similar manifestations were found to have basophilic adenomas of the pituitary (Cushing's syndrome). They believe that surgical removal of the adrenal tumor or partial resection of hyperplastic adrenal cortex may be helpful. Syndromes such as described above are considered by some to be hypophyseal in their origin with excess of adrenotropic and gonadotropic hormones. In these types and in the adrenal syndromes of both sexes before puberty such as virilism, pseudohermaphroditism and pubertas precox, Howard and Grollman¹⁵ can find no evidence that the cortical hormone per se is responsible for these changes. Kendall⁶ reconciles some of these diverse views by suggesting that the manifestations noted in these patients may be in the nature of dysfunction of the cortical hormone rather than mere overproduction of normal hormone.

The question of replacement therapy in gonadal hypofunction is undoubtedly of greater practical interest. In this category the few positive clinical results we have observed have been found to occur in individuals with concomitant signs suggesting insufficiency of adrenal function. This does not necessarily imply that asthenia or undernutrition was a prerequisite. The most marked stimulation characterized by increased libido and erection occurred in a heavily insured middle aged male who had hypotension and mucous membrane pigmentations. Associated signs of gonadal stimulation along with general bodily vigor and improvement in hypotension were also noted in several young married women. Hartman, Beck, and Thorn,¹⁸ have also described the increased sexual vigor attendant upon bodily and mental improvement after cortical therapy in patients who presented manifestations of functional derangement of the adrenal gland.

Interrelationship among the pituitary, adrenal and gonads are exemplified in such syndromes as Simmond's cachexia. A clearer insight into this endocrine disorder has been gained due to recent experimental observations upon the adrenotropic and gonadotropic hormones of the pituitary. The conclusion of most investigators is that severe pituitary deficiency causes atrophy of the adrenal cortex. In a carefully controlled series of experiments in rats Shumacker and Fuor¹⁹ showed that compensatory hypertrophy of the remaining adrenal did not occur if unilateral adrenalectomy was done in hypophysectomized animals. Stunting of growth, inactivity and lowered body temperature resulted from either pituitary or adrenal deprivation. Pituitary transplants were effectual in pituitary deprivation and adrenal cortical extracts for adrenal insufficiency. Pituitary transplants were not effectual if entire adrenal tissue was absent. The atrophy of the reproductive system seemed to be related to the absence of the gonadotropic hormone of the pituitary in both the hypophysectomized and adrenalectomized animals. In the light of such observations and numerous clinical and pathological studies the mechanism of such syndromes as Simmond's cachexia becomes reasonably clear. The low basal metabolism and blood sugar, the hypoplasia of the circulatory system with hypotension are all apparently related to the adrenal failure. These subjects respond to extracts derived from the pituitary and adrenal cortex. They are apt to react poorly to insulin very much as do cases of Addison's disease. As a matter of fact the use of the adrenal cortex extract is gently indicated in the late stages of the disease when profound asthenia and prostration indicate a failing if not already absent adrenal function. Evidence in this direction was derived from the data of a woman aged forty six years* (service of Dr. J. C. Doane) who had presented the complete clinical picture of Simmond's cachexia. At necropsy the adrenals were replaced by unrecognizable fragments of tissue containing some chromaffin framework.

In their incipience particularly in early life these syndromes may not be well defined and are apt to present trying problems of diagnosis and management. The following patient (the subject of a fuller report by Wohl and Ettelson) is an example of this type.

*The complete details of this case are described by Dr. Doane and Dr. Gouley in a forthcoming report.

J. C., a female, aged sixteen years had noticed failing appetite and strength during the past year. The present weight was 82 pounds which represented a loss of 25 pounds during the same period. There was a cessation of menstruation. Pallor was noted though the blood count showed only a moderate lowering of hemoglobin to 13.5 gm. per 100 c.c. with a normal red cell count. Pulse rate was 44 to 50 per minute, blood pressure was 82/70; x-ray showed hypoplasia of the heart and large vessels, basal metabolic rate was minus 34 per cent with a blood cholesterol of 145 mg. per 100 c.c. Blood sugar 87 mg. per 100 c.c. with a fairly normal glucose tolerance, though highest peak of curve was at 150 mg. She had none of the skin changes of Simmond's disease but showed a slight bluish pigmentation of lips and roof of the mouth. Dietetic management and thyroid medication had no effect. Upon three injections a week of adrenal cortex extract (25 gm. per 1 c.c.) in doses of 1 c.c., she improved in strength, mental outlook and gained 12 pounds in about three months. Blood pressure increased only slightly from an average of 80/60 to 90/70.

Another young female in a similar category with the added feature of allergy was also observed. The patient was an unmarried female aged twenty-four years who though 5 feet 2 inches in height weighed only 80 pounds. She suffered from asthenia and anorexia. In addition bronchial asthma had been present since childhood. There was a sensitivity to milk and wheat proteins verified by intradermal reactions and she was symptomatically sensitive to various meats though not strikingly reactive to skin tests. She showed a general underdevelopment of secondary sex characteristics; blood pressure was 95/55; basal metabolism rate was -20 per cent. She slowly gained 10 pounds in about four months under treatment with cortical extract though not receiving the extract regularly. During this period the asthma was definitely improved. In this regard the recent work of Wolfram and Zwemer²⁰ is of interest in that it apparently lends some support to the clinical application of cortical hormone in allergic states. In egg albumen anaphylaxis in normal guinea pigs, they found a greater proportion of survivals in those receiving cortical hormone, particularly when given two to six hours before the shocking dose.

VITAMIN METABOLISM

The uncontradicted evidence that the adrenal gland is a rich source of vitamin C has furthered the thought that the adrenal gland (particularly the cortex) was in some way connected with vitamin metabolism. It was found, for example, that changes occurred in some avitaminoses—atrophy in avitaminosis A and hypertrophy in avitaminosis B and C. Some investigations appeared to have established the efficacy of adrenal extracts in the treatment of deficiency due to deprivation of vitamins B and C. The dependence of the contained vitamin C upon dietary factors was noted by Siehrs and Miller²¹ who found that ascorbic acid promptly disappeared from the adrenals of guinea pigs on a scorbutic diet and reappeared when adequate amounts of orange juice were added to the diet. Recently Baena²² found that albino rats could run longer in an exercise apparatus if treated with adrenal cortex extract and ascorbic acid than if injected with cortical extract only. The whole situation has been critically reviewed by Grollman and Firor.²³ They found that under properly controlled conditions a purified cortical extract had no protective effect in animals suffering from scurvy or vitamin B₁ deprivation. Likewise ascorbic acid had no ameliorating effect upon the course of adrenal insufficiency in rats.

Though these results modify pre-existing views on the subject, the logical presentation of the authors and the controlled conditions of the study lend weight to the supposition that the connection between the adrenals and vitamins is not a direct one. On the other hand the absolute exclusion of the vitamin under investigation does not pertain to most human deficiencies. The

possibility that under such partial deprivation the cortical hormone or some unknown substance contained in the extracts of the gland may condition the amount of vitamin necessary or aid in its utilization, as suggested by Lockwood and Hartman,²⁴ cannot therefore be altogether ignored. There is some resemblance also in the susceptibility to infections and intoxications exhibited by adrenalectomized rats and the similar susceptibility in clinical and experimental scurvy. Comparable thoughts concerning the interrelations of hormones and vitamins have been recently expressed in an editorial of the *Journal of the American Medical Association*.²⁵ As an incidental observation we have repeatedly noted in adrenalectomized rats a soreness of the inner canthus of the eye (not xerophthalmia) on diets apparently ample in vitamin B complex for normal rats. The only direct effect which we have noted clinically was in the patient described under the heading of postoperative edema. In this patient a definite pellagrous like eruption disappeared within a few days after cortical hormone administration and before any other change in diet or therapeutics had been instituted. Whether a similar effect could be obtained with the refined hormone which we are now using, it is not possible to state at this time.

POSTOPERATIVE NUTRITIONAL EDEMA

Occasionally edema which is not related to cardiac or renal disease appears in postoperative patients. As pointed out by Jones and Eaton,²⁶ the edema appears to belong to the category of nutritional deficiency and is usually accompanied by hypoproteinemia. In their series it appeared after surgical operations upon the gastrointestinal tract and in patients with definite evidence of sub-nutrition. Usually large quantities of fluid had been given parenterally after operation in the form of salt solution. We have had occasion to note this syndrome as a postoperative complication and in each instance there has been a definite concomitant state of nutritional deficiency. An editorial in the *Journal of the American Medical Association*²⁷ emphasizes the probable pathologic shift of fluids and electrolytes in such conditions. The possible therapeutic benefit of restoring such shift by adrenal cortex extract was suggested by the events in the following case history:

H. I. R., female, aged forty-two years (service of Dr. R. Boyer and Dr. J. C. Doane), following a long period of Sippy diet was operated upon. The laparotomy revealed calculous cholecystitis and a cholecystectomy was performed. For several weeks thereafter she remained in a state of profound asthenia and with a blood pressure as low as 60/50. Intravenous injections of glucose and saline solutions were used but an increasing edema was noted. A scaly dermatitis of the neck and arms somewhat resembling a pellagrous eruption appeared. The blood urea nitrogen about one month after operation was 116 mg. per 100 c.c.; blood sugar, 69 mg.; chlorides 420 mg. per 100 c.c. (whole blood); the blood count revealed a moderate secondary anemia. Unfortunately serum proteins were not done.

At this time adrenal cortex extract was started in daily dosage of 2 c.c. (1 c.c. = 30 gm. of whole gland). Within several hours after the first injection the blood pressure was 80/60 but fell back again to 60/50 the next day. Subsequently the systolic blood pressure varied between 90 and 100 mm. of mercury. The edema and the pellagrous like eruption disappeared and the patient was strong enough to be discharged about one week after the inception of adrenal cortex injections. She succumbed later at home to a subphrenic sup-puration. The significant data while under treatment are summarized in Table I.

TABLE I

DATE	ADRENAL CORTEX EXTRACT	B.P. MM. OF HG.	UREA N. MG.
12-2	2 c.c.	60/50	116
2 hours later		80/60	
12-3	2 c.c.	60/50	
12-4	2 c.c.	90/70	100
12-5	2 c.c.	100/60	61
12-6	2 c.c.		
12-7	2 c.c.	100/65	33
12-7	2 c.c.	100/60	29

BLOOD

There are comparatively few reports regarding the hematologic features of adrenal insufficiency. Corey and Britton²⁸ noted that in cats the red cells after adrenalectomy increased from an average of 9,800,000 to 15 million per c. mm. In the light of the observations already mentioned, one would be inclined to relate this change to hemoconcentration with resultant higher cell-plasma ratio. However there was also noted a fall in the leucocyte count mainly affecting the neutrophiles which could be raised again by the injection of cortical extract. It has been occasionally suggested that the use of adrenal cortex extract may be effective in agranulocytic syndromes. Fitz-Hugh²⁹ has recently commented on this possibility. We had occasion to study the effects of this substance in two young women and one young man during a granulopenic phase in the course of infectious mononucleosis. In one patient, a nurse, membranous lesions in the mouth promptly disappeared though no demonstrable effect on the blood picture was noted. In the second patient small aphthous ulcers also cleared up, asthenia improved, but the abnormal blood picture persisted for many weeks thereafter. Since the administration of the extract in each case was limited to a few injections it is not possible to estimate the effect of long continued administration. The possibility is suggested that the hormone may complement or condition some function ordinarily assumed by granular leucocytes and in the absence of which the necrotic lesions so characteristic of agranulocytic processes are noted. This thought seems to be borne out by the work of Szent-Györgyi³⁰ who believes that the adrenal cortex is connected in some way with the oxidizing mechanism of the peroxidase system.

INFECTION

The susceptibility of adrenalectomized animals to infections and intoxications has led experimenters to investigate the possibility that adrenal function is an essential link in the immunity mechanism. Hartman and Scott,³¹ using adrenalectomized rats, believe they have demonstrated that adrenal cortex extract exerted a definite protective effect against the chronic intoxication produced by suspensions of dead staphylococci and the acute intoxication induced by dead typhoid bacilli. Marmorston-Gottesman and Perla³² found that bilateral adrenalectomy in Wistar strain rats lowered the natural resistance to infections with *Bartonella muris*. However the acquired immunity due to a previous infection was not altered by a later adrenalectomy.

We have verified the observations of Hartman and Scott with respect to the acute intoxication produced by dead typhoid bacilli (Fig 1). In addition we have found that adrenalectomized rats are not only incapable of withstanding the large doses of dead typhoid bacilli used in these experiments but that they also succumb during attempts at immunization with repeated graded doses of vaccine, in contrast to the survival of adrenalectomized rats treated with adrenal cortex injections. There is an obvious fallacy in applying these observations to clinical infections. If the whole situation is merely the protective or replacement effect of the hormone in an animal thrown into insufficiency, then there is no true counterpart to the ordinary course of events

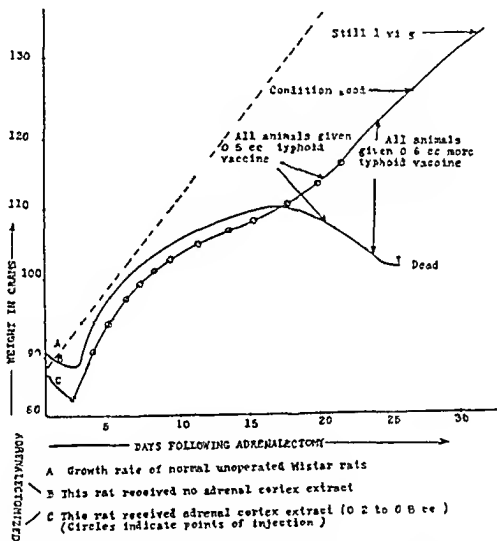


Fig 1—Protective effect of adrenal cortex extract against typhoid vaccine intoxication in adrenalectomized rats

in a clinical infection. On the contrary if adrenal insufficiency could be shown to occur in severe or protracted infections, there would be some logic in the application of these experimental observations to the practical therapy of human infections.

There are pathologic observations which suggest this possibility. Dietrich³³ has described edema of the adrenal cortex in protracted infections, corresponding to the severity of the infection there may be noted degenerative changes in the lipoids of the outer layers of the fascicular zone, the glomerular zone and to a lesser extent, the reticular layer. Asehoff³⁴ states that these changes are particularly noteworthy in diphtheria, scarlet fever and septic infections particularly those due to streptococci. Fig 2 illustrates such a case

from our own series. Robbins³⁵ reported the case of a young woman who developed marked signs of adrenal insufficiency during a streptococcic infection of the throat. The prompt amelioration of these manifestations after the use of adrenal cortex extract (Hartman) seemed to be more than coincidental. If the protection exhibited by the hormone could be shown to be due to actual reinforcement of the immune mechanism, this might also lend credibility to reports upon the favorable effect of adrenal cortex extract in infections. Whitehead and Smith³⁶ have reported upon the use of the extract



Fig. 2.—Widespread vacuolization and destruction of the fascicular zone of the adrenals in a young woman suffering from a long-standing streptococcic infection.

in the treatment of five patients with various infections, one of which was typhoid fever. He believed that some benefit was detected. Our own experience in this category has been limited to a few patients suffering with staphylococcus aureus bacteremia and in several patients with infectious (atrophic) arthritis. In the latter group one patient particularly illustrates the necessity for selecting the appropriate type of case.

I. M., male, aged fifty-two years, suffered with a fairly generalized and disabling atrophic arthritis which appeared subsequent to gallbladder surgery for calculous cholecystitis. There was a swarthy pigmentation which was general and not particularly characteristic of Addison's disease. However, anorexia, malnutrition, a blood pressure of 70/55 and the pres-

case of healed fibroid pulmonary tuberculosis suggested the necessity for adrenal cortex administration especially since dietetic and other measures including vaccines gave no improvement. On injections of 1 cc (30 gm whole gland) twice a week for eight weeks, appetite improved, there was a gain in weight of 12 pounds, and the blood pressure remained about 110/70 at discharge from the hospital. With weekly injections at home the patient after several more months reached a weight of 110 pounds, representing a total gain of 20 pounds, blood pressure 125/80 and a surprising improvement in the arthritis.

Comment—Though there was present an apparent indication for the use of the hormone in this patient and possibly in others we have seen, its indiscriminate use in unselected cases is obviously futile. Since clinical observations are fraught with the usual uncertainty common to uncontrolled conditions, we must await further confirmation of results which so far are merely suggestive.

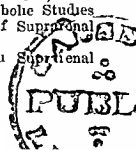
SUMMARY

Wider use of adrenal cortex therapy may be expected due to recognition of mild or subclinical variants of Addison's disease especially if significant changes in blood electrolyte pattern can be demonstrated. Similar indications are found in certain pluriglandular disturbances such as Simmonds's cachexia and other disorders involving adrenal interrelations with pituitary and gonads.

The use of adrenal cortex in other conditions not so clearly demarcated is discussed. These include particularly shock, vitamin deficiency syndromes and infections. Emphasis is placed upon individualization by selection of proper material and the presence of changes suggesting the need of adrenal cortical hormone.

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STUDIES ON YEASTLIKE FUNGI ISOLATED FROM PULMONARY DISEASE (BRONCHOMONILIAS)*

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INTRODUCTION

IT IS now generally recognized that a number of diseases are caused by fungi. Recently the yeastlike fungi grouped under the name of monilia are stimulating increasing interest as the etiologic factor in various diseases. Progress in their study, however, has been hampered by the lack of dependable methods of classifying them into their respective species.

HISTORICAL

The first mention of the name monilia in the literature was made by Persoon (1797 and 1801) who applied it to a group of twelve fungi which he placed into this genus. The next mention was made in 1839 when Langenbeck isolated the organism which we now recognize as *Monilia albicans* from a case of thrush. Since then, the name monilia has occurred in the literature from time to time, frequently under other names, such as, *saccharomyces*, *parasaccharomyces*, and *mycoterula* (Gay, 1935). Monilias have been isolated from vaginal secretions, skin infections, bone infections, abscesses, gastrointestinal tract, respiratory tract, maxillary sinuses, thrush and sprue. Ashford (1917) has reported the isolation of a certain monilia from one hundred cases of sprue which he has called *Monilia psilosis*.

Bronchomoniliasis is the name given to infections of the respiratory tract caused by any of the organisms of the genus *Monilia*. The first case of this kind was reported by Castellani in 1905 in Ceylon, India. The patient was employed by a Ceylon firm as a "tea taster" and the disease was referred to as "the tea taster's cough". In the United States the first case reported was one by Boggs and Pineoffs (1915) of Baltimore. Since then, many other cases have been reported (Warr, 1931; Wannock, 1936; Haythorn and associates, 1932; Baskin and associates, 1934; Tanner and Tanner, 1927). The largest group was reported by Flinn, Flinn and Flinn (1935) of Prescott, Arizona, who described nine cases. Recently Ikeda (1936) reported the finding of monilia in specially stained sections of fatal respiratory diseases and in which the organism had been missed in previous examinations.

Classification of these yeastlike organisms (monilia) into species is a rather difficult problem because the methods which are available are not entirely reliable. Carbohydrate fermentation and agglutination tests have been used most extensively. Both have, however, been found unsatisfactory (Brenhan, 1931; Wachowiak and associates, 1934; Lamb and Lamb, 1933, 1935; Hines, 1924).

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Organisms which may ferment certain carbohydrates at one time may lose, or gain, this ability temporarily or permanently. Agglutination tests have been found unreliable because the organisms sometimes aggregate spontaneously, probably because of their large size.

Stone and Garrod (1931) studied precipitin tests with monilias using saline heat extracted antigens. They believed that the results obtained in these precipitin tests were consistent. More recently, Lamb and Lamb (1933 and 1935) offered a method for precipitin tests using a carbohydrate fraction of the organism as the antigen. The results of these tests appeared to be quite specific and stable. They have also presented a carbohydrate utilization test employing seven carbohydrates, i.e., dextrose, levulose, maltose, sucrose, xylose, galactose, and inulin. This method also appears to be quite reliable.

My interest in the study of yeastlike organisms (monilia) began in 1933 in Prescott, Arizona, when a fatal case came under the care of Flinn, Flinn, and Flinn with whom I am associated. Monilia had been isolated and identified morphologically and culturally from the patient's sputum previous to his death. At autopsy yeastlike organisms were isolated from the brain and the apices and bases of both lungs. Attempts to classify these organisms into their species were based entirely upon acid and gas production from carbohydrates according to Castellani (1928) and according to him were found to be *M. pinoyi*. Upon reviewing the literature on this subject, it was learned as indicated above that this is not a reliable method since the ability to ferment carbohydrates is a variable one.

OBJECT AND SCOPE

This work was undertaken with the hope of finding a more rapid and reliable method of classifying monilias. Certain strains of monilia have been tested repeatedly (1933, 1934, 1935, and 1937) for their constancy of behavior towards carbohydrates. This work includes the carbohydrate utilization tests, as suggested by Lamb and Lamb (1933, 1935) and precipitin tests with carbohydrate fraction antigens. Agglutination tests were also studied.

METHODS

1. *Isolation of the Organisms from Sputum.*—The following method was used in isolating the organisms from the sputums of suspected cases of bronchomoniliasis.

The patient was given a sterile jar, a mouth wash, and a bottle of sterile water. The mouth wash was a very dilute solution of bichloride of mercury. The patient was instructed to sterilize his mouth and throat with this solution, then to thoroughly rinse his mouth and throat with the water and expectorate any coughed up sputum directly into the sterile jar. If possible the morning sputum was obtained.

The isolation of the organisms in the laboratory was accomplished in two ways: (1) White "flecks" in the sputum were picked out and inoculated on Sabouraud's agar slants or plates. (2) The sputum was mixed with an equal volume of 70 per cent alcohol, and allowed to stand for one to one and one-half hours. Sabouraud's dextrose agar slants or plates were then heavily inoculated with the sterilized sputum.

Both methods yielded equally good results. A visible growth of the organism could be expected in from one to three days. To obtain pure cultures, a saline suspension of a characteristic colony was made, this was streaked over Sabouraud's agar plates. On these plates, the characteristic round, smooth, raised and pitted colonies can be easily distinguished from the contaminants, which are usually pigmented staphylococci. A morphologic and cultural study of eleven organisms from known pulmonary diseases and from two probable cases (Nos. 19 and 21) will be found in Figs. 1 to 4 and in Table I. Organisms numbers 7, 8, 9, 14, 15, and 22 were isolated from 178 consecutive sputum specimens from cases of pulmonary diseases.

2 *Isolation of Monilias from Normal Throats*—Cultures were made from 100 students at Stanford University in this experiment. The throat of each student was swabbed with sterile cotton and streaked on Sabouraud's agar. The plates were incubated at 37° C. for several days and then examined for yeast-like organisms. Table I shows the morphologic and cultural characteristics of the three species of the organisms obtained in this experiment. Their numbers are 10, 11, and 12.

3 *Carbohydrate Fermentation and Carbohydrate Utilization Tests*—A. For regular carbohydrate fermentation tests, Durham fermentation tubes were prepared, using a 1 per cent concentration of the carbohydrate (c.p.) in meat infusion broth. The tubes were inoculated from a twenty-four hour culture growing on Sabouraud's agar, and incubated at 37° C. They were incubated for thirty days or until acid and gas appeared. The following carbohydrates were used: dextrose, levulose, maltose, d. galactose, sucrose, mannite, lactose, dextrin, rhamnose, arabinose, adonite, inulin, sorbite, starch, glycerine, dulcitol, inositol, silicin, xlose, raffinose, and erythrite. In the dextrin broth the fungi developed extensive mycelia. The results of the tests when the carbohydrate was attacked are found in Tables II, III, IV, and V. All tests were made in duplicate and stained smears were made on all positives to rule out contamination. If any disagreement was found the test was repeated.

B. Carbohydrate utilization tests were carried out with dextrose, levulose, maltose, galactose, xlose, sucrose, and inulin. Tubes containing 1 per cent of the individual carbohydrates were inoculated as indicated under A above and incubated for thirty days at 37° C. At the end of the thirty day period, eight to ten drops of each of the media were tested for the utilization of the carbohydrates with Benedict's qualitative solution. Sucrose and inulin were first hydrolyzed with an equal number of drops of dilute hydrochloric acid before testing with Benedict's solution. The results are recorded in Tables IV and V, utilization being indicated by ⊕ and +.

4 *Preparation of Precipitating and Agglutinating Serums*—Antiserums were prepared by injecting rabbits with heavy saline suspensions of the organisms. Five heavy loops from a twenty-four-hour culture of the organism on Sabouraud's agar were added to 10 c.c. of saline with 0.5 per cent phenol and incubated for four or five days at 37° C. to kill the organisms. The injections were made in three series of three doses at weekly intervals. On the first day 1 c.c. was injected, on the second day 2 c.c., and on the third day 5 c.c. Six days

TABLE I
CULTURAL AND MORPHOLOGIC STUDIES

SOURCE	STRAIN NO.	GRAM REACTION	ODOR	PLAIN BROTH	AGAR	LEAD ACETATE	RATE OF GROWTH	LITMUS MILK	GELATIN	PATHOGENICITY	GIANT COLONIES	YEARS STUDIED
Sputum	1	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	Rabbit	+	1933 to 1937
Rabbit 1	1A	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	
Brain	1B	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	
Left lung	1C	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	
Right lung	1D	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	
Sputum	2	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	Rabbit	+	
Rabbit 2	2A	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	
Sputum	3	+	Yeasty	Sedi- ment	White pasty	-	Slow	-	Inverted Pine Tree	Rabbit	Slow	1934 to 1937
Sputum	4	+	Yeasty	Sedi- ment	White pasty	-	Rapid	-	Inverted Pine Tree	Rabbit	+	1934 to 1937
Sputum	5	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	Rabbit	+	1934 to 1937
Sputum	7	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	Rabbit	+	1935 to 1937
Sputum	8	+	Yeasty	Sedi- ment	White pasty	-	Rapid	-*	Inverted Pine Tree	Rabbit	+	1935 to 1937
Sputum	9	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	Rabbit	+	1935 to 1937
Normal throat	10	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	Rabbit	+	1935 to 1937
Normal throat	11	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	1935 to 1937
Normal throat	12	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	1935 to 1937
Sputum (?)	13	+	Yeasty	Sedi- ment	White pasty	-	Medium	-*	Inverted Pine Tree	0	+	1936 to 1937
Sputum	14	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	Rabbit	+	1936 to 1937
Sputum	15	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	Rabbit	+	1936 to 1937

+ Indicates positive.
- Indicates negative.

0 Indicates not done.
* Indicates slightly alkaline (1937).

TABLE I—CONT'D

SOURCE	STRAIN NO	CULM REACTION	ODOR	EXAM IN BOTT	APPEAR	REACTANT	RATE OF GROWTH	INITIAL MILK	RELATIVE	PATHOGENICITY	GIANT COLONIES	YEARS STUDIED
Thrush	16	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	1936 to 1937
Mouth	17	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	1936 to 1937
Mouth	18	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	1936 to 1937
Another Rab	19	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	1936 to 1937
Mouth	20	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	1936 to
Another Rab	21	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	0	+	1936 to
Sputum	22	+	Yeasty	Sedi- ment	White pasty	-	Medium	-	Inverted Pine Tree	Rabbit	+	1936 to 1937

TABLE II
1933 BIOCHEMIC REACTIONS

SOURCE	STRAIN NUMBER	DEXTROSE	LEVULOSE	MALTOSE	D GALACTOSE
Sputum	1	⊕	⊕	⊕	++
Rabbit 1	1A	⊕	⊕	⊕	++
Brain	1B	⊕	⊕	⊕	++
Left lung	1C	⊕	⊕	⊕	++
Right lung	1D	⊕	⊕	⊕	++
Sputum	2	⊕	⊕	⊕	++
Rabbit 2	2A	⊕	⊕	⊕	++
Sputum and mouth	6	⊕	⊕	⊕	++

⊕ Indicates acid with gas + Indicates acid - Indicates no acid or gas

TABLE III
1934 BIOCHEMIC REACTIONS

SOURCE	STRAIN NUMBER	DEXTROSE	LEVULOSE	MALTOSE	D GALACTOSE
Sputum	1	⊕	⊕	⊕	+
Rabbit 1	1A	⊕	⊕	⊕	+
Brain	1B	⊕	⊕	⊕	++
Left lung	1C	⊕	⊕	⊕	+
Right lung	1D	⊕	⊕	⊕	+
Sputum	2	⊕	⊕	⊕	+
Rabbit 2	2A	⊕	⊕	⊕	+
Mouth and sputum	6	⊕	⊕	⊕	+
Sputum	3	⊕	+	⊕	++
Sputum	4	⊕	⊕	+	++
Sputum	5	⊕	+	⊕	++

⊕ Indicates acid with gas + Indicates acid - Indicates no acid or gas

after the last dose, the rabbits were tested. If satisfactory titers of precipitins and agglutinins were present the rabbits were bled.

5 *The Precipitin Test.*—A. *Preparation of Antigen:* Each organism was cultivated on Sabouraud's agar in Petri dishes heavily inoculated for forty-eight hours. The growth was scraped off with a sterile glass slide and emulsified in 10 c.c. of sterile saline. Five-tenths cubic centimeter normal hydrochloric acid was added and allowed to set for twelve hours with occasional shaking. Thereafter the mixture was placed in a water-bath at 100° C. for fifteen minutes. It was then centrifuged and the supernatant liquid drawn off. This was neutralized with normal sodium hydroxide and centrifuged again. The supernatant liquid, which served as the antigen, was then carefully drawn off. Benedict's qualitative solution indicated the presence of reducing sugars. The pH during extraction was 4.8. The antigens showed an average nitrogen content of 5.39 mg per c.c.

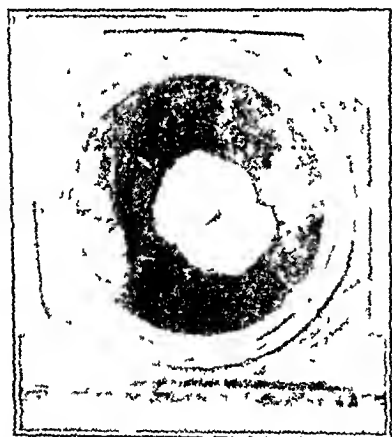


Fig. 1



Fig. 2

Fig. 1—Two-weeks-old growth of *Monilia albicans* (*M. pinoyi castellani*) on Sabouraud's dextrose agar

Fig. 2—One-month-old growth of *Monilia albicans* on Sabouraud's dextrose agar.

B. *Precipitin Test:* The following antigen dilutions were employed: $\frac{1}{10}$, $\frac{1}{20}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{1200}$, $\frac{1}{1400}$, and $\frac{1}{1500}$. Three-tenths cubic centimeter of each of the dilutions was carefully layered over 0.3 c.c. of the serum. Controls with a normal rabbit serum were used. The tests were read for precipitin ring formation after five minutes and were then incubated at 37° C. for one hour, after which they were read again and placed in the icebox overnight. Final readings were made in the morning. The results are given in Table VI

C. *Precipitin Absorption Test:* To five cubic centimeters of each of the precipitating serums was added 5 c.c. of a heavy suspension of the organisms, and the mixture was shaken for one-half hour at room temperature. The individual mixtures were then placed in the incubator for one hour at 37° C. with occasional shaking. Following this, they were stored in the ice box overnight, centrifuged and the supernatant fluid constituted the absorbed serum. Each absorbed serum was tested against its homologous antigen in one-half dilution for residual precipitating antibody. If complete absorption had taken place, the

absorbed serums were run against all of the antigens in a 1:2 dilution. If antibodies remained the absorption was repeated. Each of the twenty-two strains studied gave no precipitate when tested with each of the nine absorbed serums.

6 Agglutination Tests—A Preparation of Fungus Suspensions. To eighteen-hour cultures of the organisms on Sabouraud's agar slants, 10 c.c. of saline were added. The growth was scraped off with an inoculating loop and well suspended. About 8 c.c. of each suspension were placed in a sterile test tube. The suspensions were well shaken for one half hour. Five tenths cubic centimeter of each of the following dilutions of the antiserums was prepared: $\frac{1}{10}$, $\frac{1}{20}$, $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{1200}$, $\frac{1}{1400}$, $\frac{1}{1500}$. Five tenths cubic centimeter of the organism suspension was added to each serum dilution. These were mixed well and incubated for two hours at 37° C. The tests were read and incubated overnight in the ice box. Final readings were made in the morning. The results of these agglutination tests are given in Table VI.



FIG. 3

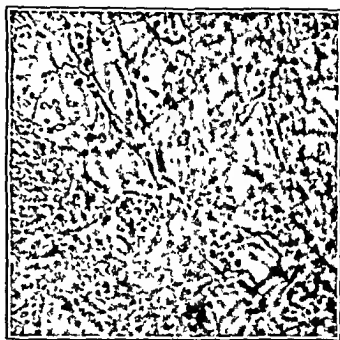


FIG. 4

Fig. 3—A twenty-four hour growth in dextrin broth. Note the yeastlike budding cells.

Fig. 4—A four weeks old growth in dextrin broth. Note the extensive mycelial development.

B Agglutination Absorption Test. To 5 c.c. of a 1:10 dilution of serum, 5 c.c. of a very heavy suspension of the organism were added. This mixture was shaken for one half hour and incubated at 37° C. for two hours with occasional shaking. It was then placed in the ice box overnight, centrifuged and the absorbed serum drawn off. Each serum was tested with its homologous organism for completeness of absorption. If this was incomplete, the absorption was repeated. If the absorption had been complete, 0.5 c.c. of the absorbed serum was added to each tube containing 0.5 c.c. of the organism to be tested. It is important especially with monilia to have saline controls of each of the organisms as well as normal rabbit serum controls, because of the tendency to spontaneous agglutination. The results of the agglutination absorption tests of the 22 strains with each of the nine absorbed serums were negative, that is no agglutination took place.

DISCUSSION

A study of Table I reveals that the monilias used in this study are from four main sources: (1) Sputum in pulmonary diseases. (2) Autopsy materials No. 1A, 1B, 1C, 1D, and 2A. (3) Normal throats. (4) Minor upper respiratory conditions.

Eleven of these organisms are definitely known to have their origin from sputums in cases of pulmonary disease; two others, No. 19 and 21, are probably also from pulmonary diseases, as indicated by the information obtained with the cultures. Six of the 11 definitely known organisms isolated from pulmonary diseases (No. 7, 8, 9, 14, 15 and 22) were obtained in the examination of 178 consecutive cases of pulmonary infections. This result indicates that 2.93 per

TABLE IV
1935 BIOCHEMIC REACTIONS AND CARBOHYDRATE UTILIZATION

SOURCE	STRAIN NUMBER	DEXTROSE	LEVULOSE	MALTOSE	D-GALACTOSE	SUCROSE	XYLOSE	INULIN	LACTOSE	DENTRIN	L-ARABINOSE
Sputum	1	⊕	⊕	⊕	+	+	+	-	-	+	-
Rabbit 1	1A	⊕	⊕	⊕	+	+	+	-	-	-	-
Brain	1B	⊕	⊕	⊕	+	+	+	-	-	-	-
Left lung	1C	⊕	⊕	⊕	+	+	+	-	-	-	-
Right lung	1D	⊕	⊕	⊕	+	+	+	-	-	-	-
Sputum	2	⊕	⊕	⊕	+	+	+	-	-	-	-
Rabbit 2	2A	⊕	⊕	⊕	+	+	+	-	-	-	-
Sputum	3	⊕	⊕	⊕	+	+	+	-	-	-	+
Sputum	4	⊕	⊕	+	+	+	+	-	-	-	-
Sputum	5	⊕	+	+	+	+	+	-	-	-	+
Sputum	7	⊕	+	+	+	+	+	-	-	-	-
Sputum	8	⊕	+	+	+	+	+	-	+	+	-
Sputum	9	⊕	⊕	+	+	+	+	-	-	-	-
Normal throat	10	⊕	⊕	+	+	+	+	-	-	-	-
Normal throat	11	⊕	⊕	+	+	+	+	-	-	-	-
Normal throat	12	⊕	⊕	+	+	+	+	-	-	-	+

⊕ Indicates acid and gas, and carbohydrate utilization.

+ Indicates carbohydrate utilization and acid.

- Indicates no acid or gas.

* Indicates not completely.

cent of all pulmonary diseases may be caused by monilia. In two instances both monilia and *Mycobacterium tuberculosis* were found in the same sputum. The monilias thus found are No. 3 and 6. (No. 6 was lost in 1934.) These two organisms are not included in the six mentioned above as found in 178 sputum examinations. The patients in whose sputums both of these organisms were demonstrated grew progressively worse in spite of the best care and treatment possible. This indicates that a mixed infection of monilia and *Mycobacterium tuberculosis* is a very serious combination.

Three monilia cultures (No. 10, 11, 12) were obtained from the throats of 100 apparently normal individuals, indicating that monilias are found in the throats of 3 per cent of normal persons without causing any apparent ill effects. The organisms recovered from cases originating in minor upper respiratory conditions are No. 13, 16, 17, 18 and 20.

The results of the cultural and morphologic studies of all the moniliae recorded in Table I, show practically no difference in either of these characteristics. The only differences observed were in the cases of No 2, 8, and 13 which produced a very slight alkalinity in litmus milk on one occasion (1937) and a variation in the rate of growth of some of these strains, No 3 and 18 being rather slow, while No 4 and 8 were rather rapid in their development.

The ability of the organisms to attack carbohydrates with acid and gas production and acid production alone is demonstrated in Tables II, III, IV, and V. A study of these tables reveals the fact that some of the organisms varied in this respect at the time of their isolation i.e., No 1, 1A, 1B, 1C, 1D, 2, 2A, 3, 4, 5, and 6, while No 7 to 12 inclusive showed variations in subsequent tests. No 13 to 22, inclusive, were tested only once (1937) and it is there

TABLE V
1937 BIOCHEMIC REACTIONS AND CARBOHYDRATE UTILIZATION

SOURCE	STRAIN NUMBER	DEXTRSE	LEVULOSE	MALTOSE	D-GALACTOSE	SUCROSE	XYLOSE	MULIN	LACTOSE	DEXTRIN	ARABINOSF
Sputum	1	⊕	⊕	⊕	+	+	+	-	-	+	-
Rabbit 1	1A	⊕	⊕	⊕	+	+	+	-	-	-	-
Brain	1B	⊕	⊕	⊕	+	+	+	-	-	+	-
Left lung	1C	⊕	⊕	⊕	+	+	+	-	-	+	-
Right lung	1D	⊕	⊕	⊕	+	+	+	-	-	+	-
Sputum	2	⊕	⊕	⊕	+	+	+	-	-	+	-
Rabbit 2	2A	⊕	⊕	⊕	+	+	+	-	-	+	-
Sputum	3	⊕	⊕	⊕	+	+	+	-	-	+	-
Sputum	4	⊕	⊕	⊕	+	+	+	-	-	+	-
Sputum	5	⊕	⊕	⊕	+	+	+	-	-	+	-
Sputum	7	⊕	⊕	⊕	+	+	+	-	-	+	-
Sputum	8	⊕	⊕	⊕	+	+	⊕	-	-	+	-
Sputum	9	⊕	⊕	⊕	+	+	+	-	-	-	-
Normal throat	10	⊕	⊕	⊕	+	+	+	-	-	-	-
Normal throat	11	⊕	⊕	⊕	+	+	+	-	-	-	-
Normal throat	12	⊕	⊕	+	+	+	⊕	-	-	-	-

⊕ Indicates acid and gas and carbohydrates utilized

+ Indicates acid and carbohydrates utilized

- Indicates no acid or gas and carbohydrates not utilized

fore known whether or not they may vary later in this respect. The most striking variation was demonstrated by the monilia 8 which gained the ability to produce gas from levulose, maltose and sucrose and lost the ability to attack lactose with acid production. Three organisms acquired the power to produce gas from levulose (No 5, 7, and 8), while eight were able to accomplish this in maltose (No 4, 5, 7, 8, 9, 10, 11 and 12) and only two in sucrose (No 8 and 12). Dextrin, arabinose and lactose were not consistently attacked.

A study of Tables IV and V, which present results for the utilization of the seven carbohydrates, dextrose, levulose, maltose, galactose, sucrose, xylose, and mulin, in a method suggested by Lamb and Lamb (1935) reveals the fact that no variation occurred in two tests made in 1935 and 1937 and that all organisms studied utilized all but mulin and thus fall in Group 1 (Lamb and Lamb),

which is comprised of *M. albicans*, *M. psilosis* and *M. candida*. Since these organisms are pathogenic they should be considered as *M. albicans* (*M. pinoyi*, *castellani*).

An examination of Table VI reveals that satisfactory concentrations of both precipitating and agglutinating antibodies were obtained in all of the animals injected in this study, and that the titers of the antiserums for the precipitins were consistently less than those of the agglutinins. This indicates that the specific carbohydrates are not as good antibody stimulators as the proteins. This variation is most strikingly demonstrated by monilia 8. In Rabbit A70 the precipitin titer resulting was 1:50 and in Rabbit A71 it was 1:100, in these same rabbits the agglutinin concentrations obtained were 1:2000 and 1:4000, respectively. The concentration of 1:50 was the lowest obtained for the precipitin serums and 1:4000 was the highest recorded for the agglutinins.

TABLE VI
RABBIT IMMUNIZATION RESULTS

RABBIT NUMBER	STRAIN NUMBER	PRECIPITIN TITER	AGGLUTININ TITER	CONTROL NOR. SERUM	CONTROL SALINE
A58	1	1:100	1: 500	0	0
A59	1	1:250	1: 500	0	0
A60	1A	1:100	1: 500	0	0
A61	1A	1:100	1: 500	0	0
A72	1B	1:100	1: 500	0	0
A73	1B	1:100	1: 500	0	0
A62	1C	1:250	1: 500	0	0
A64	2	1:250	1:1000	0	0
A65	2	1:250	1:1000	0	0
A74	2A	1:250	1: 500	0	0
A75	2A	1:250	1: 500	0	0
A66	5	1:500	1:2000	0	0
A67	5	1:500	1:2000	0	0
A68	10	1:100	1:2000	0	0
A69	10	1:250	1:2000	0	0
A70	8	1:50	1:2000	0	0
A71	8	1:100	1:4000	0	0

Precipitin absorption tests with each of the twenty-two strains with each of the nine absorbed serums developed in this study gave negative results. Not in one instance could any sign of a precipitate be detected. This lack of precipitation indicates that they are all very closely related strains.

Agglutination absorption tests with the 22 strains with the same nine absorbed serums gave the same results as the precipitin absorption tests did. No spontaneous agglutination was observed. It is believed that the use of young cultures (not more than eighteen hours old) and thorough shaking prevents this objectionable reaction.

Agglutinating and precipitating absorption tests with known species of monilias, therefore, furnish a rapid and dependable method of classifying yeastlike fungi (monilia). A slow but also dependable method is that of testing their ability to utilize dextrose, levulose, maltose, galactose, sucrose, xylose, and inulin according to the method of Lamb and Lamb (1935).

SUMMARY AND CONCLUSIONS

The observations made during this study may be summarized as follows

1 Three per cent of apparently normal individuals in the group tested harbor monilia in their throats

2 The incidence of pulmonary disease caused by these organisms in 178 cases was 2.93 per cent

3 The ability to attack carbohydrates with acid or with acid and gas production is not a reliable criterion for the classification of these fungi

4 Carbohydrate utilization tests according to the method of Lamb and Lamb (1935) showed no variation in two tests, two years apart. The tests showed that all strains studied were identical and that they fall into Group 1 (Lamb and Lamb) and Group 2 (Stoval and Bubolz, 1932). This group is composed of *M. albicans*, *M. psilosis* and *M. candida*

5 Precipitin and agglutinin absorption tests demonstrated that the organisms were serologically identical

6 It is believed that the reliable results of the carbohydrate utilization tests combined with precipitation and agglutination reactions furnish a dependable method for the classification of these yeastlike organisms

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CORONARY DISEASE IN DIABETES MELLITUS*

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IT IS a well-known clinical fact that diabetes mellitus and disease of the coronary vessels are often associated. In 1864 Seegan¹ commented upon the occurrence of angina pectoris and diabetes in one patient. Since then; and especially during the past ten years, there has been accumulated a considerable amount of literature dealing with different aspects of this clinical problem. S. A. Levine² believes that among the disease entities that are etiologically related to coronary thrombosis, diabetes is second in importance only to a previously existing hypertension. In 500 cases of diabetic autopsies, I. M. Rabinovitch³ found that of those individuals whose diabetes lasted five years or more, 80 per cent had cardiovascular disease, regardless of the age of the patient and severity of the disease. Shields Warren⁴ states, "I have yet to see a diabetic at autopsy, or to read a protocol of a diabetic, whose disease lasted five years or more, free from arteriosclerosis, regardless of age." Blotner⁵ found that of 77 diabetic patients who came to autopsy, 45 per cent had well-marked diseases of the coronary vessels, while of 450 nondiabetics, only 21 per cent had definite lesions in the coronary arteries. Root⁶ found advanced coronary artery disease at autopsy twice as frequently in diabetics as in nondiabetics. Of 249 consecutive autopsies above the age of fifty years examined by Nathanson,⁶ there were 8.2 per cent showing extensive coronary sclerosis as compared with 52.7 per cent in the diabetic series of 100 autopsies.

The present work represents a study of the histopathologic appearance of the coronary vessels in diabetic and nondiabetic hearts of comparable age periods, and it consists of three parts:

1. Examination and histologic study of coronary vessels in 31 diabetic hearts.
2. Examination and histologic study of coronary vessels in nondiabetic hearts of comparable age periods.
3. Statistical analysis of the incidence of coronary artery disease in diabetic and nondiabetic patients at autopsies at The Mount Sinai Hospital.

*From the Laboratories of The Mount Sinai Hospital.
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Methods and Materials.—The hearts were fixed in 10 per cent formalin-saline according to the technique described by Gross, Epstein and Kugel,^{5, 8} and sections were cut from the left coronary artery about 1 cm. from its ostium; one from the left anterior descending branch about 2 cm. from the ostium; one from the right circumflex coronary artery, 1 cm. from its ostium, and one from the posterior descending branch about 1 cm. below the atrienventricular sulcus. The sections were imbedded in 10 per cent gelatin and kept in the incubator at 38° C. for twenty-four hours, followed by imbedding in 20 per cent gelatin for another twenty-four hours under similar conditions, and then cut by the frozen section method. The cut sections were stained with hematoxylin followed by Sudan III. Duplicate sections were imbedded by the usual paraffin method and stained with hematoxylin-eosin and Weigert's elastica method.

Coronary Arteries of Diabetic Hearts.—The ages ranged between forty-four and seventy-eight, and in addition, there was one patient six years of age and one of twenty years of age. There were 6 cases in the fifth decade, 9 in the sixth decade, 11 in the seventh decade, and 3 in the eighth decade. The six-year-old patient was a boy who was known to have had diabetes for two years and to have died in coma. Macroscopically, the coronary arteries of this heart were smooth and appeared normal throughout; microscopically they showed very mild sclerosis of the intima, but no deposition of fat. The twenty-year-old individual also died in coma and the duration of diabetes was not obtainable. Macroscopically, the vessels of this heart showed a few small yellowish flat plaques, but appeared normal otherwise, while microscopically, they showed only a moderate degree of sclerosis and no fat deposits.

Gross examination of the coronary vessels of the remaining 29 hearts showed mild degrees of sclerotic changes in 8 hearts and marked to extensive sclerosis in the remaining 21 hearts. Of the latter, 7 presented acute thrombotic occlusion of one of the coronary branches. Microscopic sections of the coronary vessels of all of these cases showed well-developed intimal thickening. This intimal thickening varied in width from 3 to 16 times that of the media of the corresponding vessel. The thickening consisted mainly of an increased amount of loose connective tissue, elastic tissue elements, collagen, and the deposition of lipid material, as stained by Sudan III. There were no qualitative differences found either in the different vessels of the same case or in the vessels of the different cases. Neither were there found any significant differences in the vessels obtained from the hearts of different age periods or changes that would be characteristic of the duration or severity of the diabetes. However, there seemed to be a quantitative difference inasmuch as the longer the duration of the diabetes and the older the patient, the more marked were these changes, and the eight cases which grossly presented mild alteration, showed similar changes, but to a lesser degree. These changes were more marked in the left anterior descending branch of the left coronary artery and in the right circumflex coronary artery; they were least developed in the posterior descending branch of the right coronary artery.

Coronary Arteries in Nondiabetic Hearts.—Twenty hearts removed from nondiabetic patients at autopsies, but with well-developed coronary artery disease, were selected for study, in order to compare the lesions found in these vessels with those found in the diabetic hearts of similar age periods. There

were five cases in each of the fifth, sixth, seventh, and eighth decades. The extent and character of the intimal sclerosis and the deposition of lipid material differed in no way from those found in the diabetic hearts. There was not found any single criterion or change that would make it possible to differentiate the coronary vessels of the diabetic heart from those of the nondiabetic heart with well-developed coronary artery disease.

Statistical Analysis of Seven Hundred Consecutive Autopsies.—In Table I, the incidence of coronary artery disease and diabetes mellitus in 700 consecutive autopsies at The Mount Sinai Hospital is classified according to the different decades. It is seen that the highest incidence occurs in the fifth, sixth, seventh, and eighth decades. In the 402 nondiabetic patients between the ages of forty and eighty, 128 hearts with well-developed disease of the coronary arteries were found at autopsy, an incidence of 31 per cent, while among the 29 diabetic pa-

TABLE I

AGE	TOTAL NO. AUTOPSIES	NON-DIABETICS	CORONARY SCL. IN NON-DIABETICS	DIABETES MELLITUS	CORONARY SCL. IN DIABETICS
0-10	108	107		1	
10-20	33	33			
20-30	48	48	3		
30-40	71	70	10	1	
40-50	127	124	20	3	
50-60	126	119	35	7	4
60-70	129	116	59	13	9
70-80	49	43	32	6	5
80-90	9	9	6		
Totals	700	669	165	31	18
40-80	431	402	146	29	18

tients there were 18 hearts with well-developed disease of the coronaries, an incidence of 62 per cent. That is, coronary artery disease was found twice as frequently in the hearts of diabetic as in the hearts of nondiabetic patients.

DISCUSSION

From the foregoing, it is seen that there is no essential difference demonstrable by these methods—Weigert's elastic and sudan fat stain—between vessels of diabetic hearts and those of nondiabetics with well-developed disease of the coronary vessels. It seems likely, therefore, that the pathogenesis of vascular disease found in diabetic hearts is essentially similar to the vascular disease found in the vessels of nondiabetic hearts with well-developed disease of the coronary vessels. This view is supported by the work of Earl R. Lehnher,⁹ who conducted chemical analysis of 25 diabetic aortas, 25 nondiabetic aortas, and 6 children's aortas and found that the changes were similar in the diabetics and nondiabetics, but that there is a greater deposition of lipid, a more marked change in the lipid allocation (increased proportion of the total lipid as cholesterol and a diminished proportion of the total lipid as fatty acid and as phospholipid) and a higher calcium and phosphorus content in the diabetic aorta. The cholesterol content was 8.07 gm. per cent in diabetics as compared with 4.8 gm. per cent in nondiabetics. The source of the lipid remains unknown.

Joslin¹⁰ favors the theory that the cholesterol, which is increased in the diabetic blood, is the basis of the vascular change. Leary¹¹ claims to have been able to reproduce all the stages of human atherosclerosis in the coronary arteries in the rabbit by feeding cholesterol, and he points out that such lesions do not occur spontaneously in rabbits. The lesions produced varied in different animals in their extent and severity, this variation is comparable to the differences exhibited by human beings. Leary makes the interesting observation that man is the only animal that eats both eggs and milk, the most common sources of cholesterol, throughout his entire lifetime, and man is the only animal that dies in early life from coronary sclerosis. I. M. Rabinovitch⁷ also favors the idea that excess blood cholesterol in the circulating blood is an important etiologic factor in the production of arteriosclerosis in the young diabetic. By careful determination of plasma cholesterol, he found an intimate association between cardiovascular disease and hypercholesterolemia, and he states that "deposition of cholesterol and its esters does not necessarily require a high cholesterol content but depends upon the ability of the blood to keep in solution a substance which is probably in a state of supersaturation." In this connection, it is interesting to note the work of I. Rathery and S. Doubrow,¹² who found that the lipoids in the intima are mainly derivatives of palmitic and stearic acid, while in the adventitia, derivatives of oleic acid are abundant.

SUMMARY

1 The microscopic appearance of vascular disease in the coronaries of diabetic hearts is similar to that of nondiabetic hearts with well developed disease of the coronary vessels.

2 The incidence of coronary artery disease in 29 diabetic patients at The Mount Sinai Hospital upon whom autopsies were performed was 62 per cent as compared with 31 per cent in 402 nondiabetic patients between the ages of forty and eighty.

NOTE: The writer wishes to thank Dr. Louis Gross, Director of the Laboratories at The Mount Sinai Hospital, for his many valuable suggestions.

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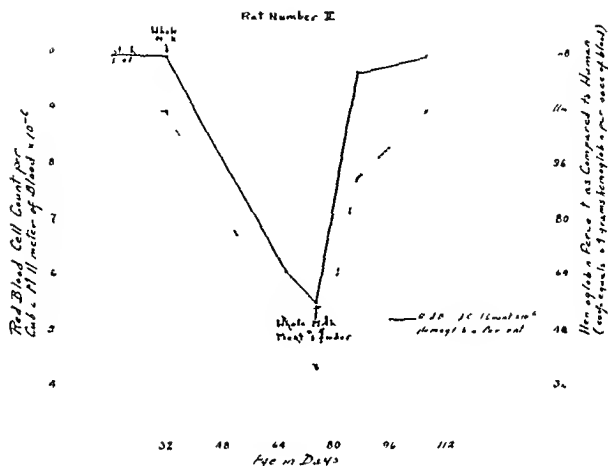


Fig. 2

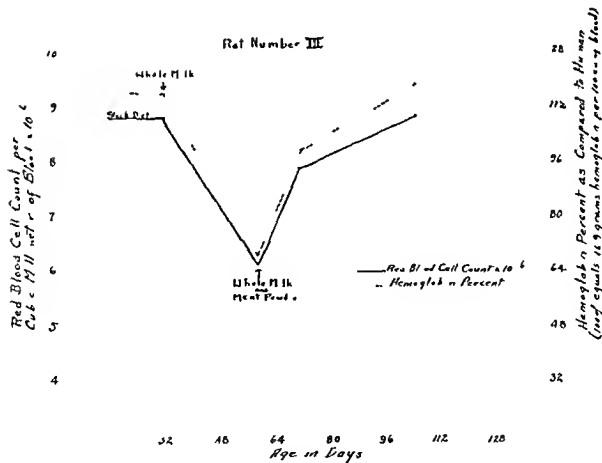


Fig. 3

RESULTS

RAT	AGE IN DAYS	WEIGHT IN GM.	DIET	DURATION OF DIET IN DAYS	RED BLOOD CELL COUNT PER C. MM. OF BLOOD	HEMOGLOBIN PER CENT AS COMPARED TO HUMAN*	CONDITION OF RED BLOOD CELLS
1	32	52	Stock	Since weaning			
	67	76	Whole milk	35	4,030,000	30	Very hypochromatic, irregular in size and shape
	75	104	Whole milk and meat powder	8	5,510,000	45	Normal
	92	136	Whole milk and meat powder	25	8,920,000	93	Normal
	101	160	Whole milk and meat powder	34	10,000,000	115	Normal
2	32	48	Stock	Since weaning			
	66	84	Whole milk	34	6,080,000	50	Hypochromatic, irregular in size and shape
	75	92	Whole milk	43	5,540,000	38	Very hypochromatic, irregular in size and shape
	87	114	Whole milk and meat powder	12	9,690,000	93	Normal
	107	160	Whole milk and meat powder	32	9,960,000	112	Normal
3	32	48	Stock	Since weaning			
	59	80	Whole milk	27	6,130,000	69	Hypochromatic, irregular in size and shape
	71	88	Whole milk and meat powder	12	7,920,000	100	Normal
	103	128	Whole milk and meat powder	36	8,900,000	119	Normal

*100 per cent equals 16.9 gm. hemoglobin per 100 c.c. of blood.

CONCLUSION

Lean beef from the round is an excellent source of iron for the treatment of secondary anemia produced by malnutrition. The iron apparently is in a readily available form.

THE EFFECT OF HIGH PROTEIN DIETS ON THE KIDNEYS OF RATS*

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RECENTLY there has been considerable controversy as to the effect of high protein diets in the human kidney. Most clinicians and especially metabolists who are in active contact with nephritic patients believe that a high protein diet will not injure the normal kidney and that such a diet plays no rôle in the etiology of the various forms of inflammatory and degenerative diseases of the kidney. High protein diets have been fed to carnivorous animals without producing anatomic kidney damage and to herbivorous animals with the production of extensive permanent renal injury. As the metabolism of the rat is similar to that of the human, this animal was chosen for the following feeding experiment.

Fifty healthy white female rats from the Wistar strain, 40 only twenty eight days old and 10 one hundred and twenty days of age, were fed a nutritionally adequate high protein diet for one hundred and fifty days. Whereas the diet of most American people contains approximately 10 per cent of the total energy value as protein calories, the following diet contains 33 per cent of the total fuel value as protein calories. An excess of food was kept in the cages at all times.

FOOD	AMOUNT IN 100 GM OF DIET	CARBON HYDRATE	PROTEIN	FAT	CALCIUM	PHOS- PHORUS	IRON
Whole wheat	50.0	27.1	5.3	0.8	0.017	0.161	0.002
Oats	25.0	22.4	5.6	2.4	0.021	0.170	0.001
Meat powder	15.0	---	13.5	0.9	---	0.083	0.003
Gelatin	5.7	---	5.2	---	---	---	---
Yeast	3.0	1.2	1.4	0.1	0.001	0.012	0.004
Calcium carbonate	1.3	---	---	---	0.520	---	---
Sodium chloride	1.0	---	---	---	---	---	---
Cod liver oil	1.0	---	---	1.0	---	---	---
Ferric citrate	0.01	---	---	---	---	---	0.003
Total	100.00	52.0	31.0	5.2	0.512	0.425	0.01
Calorie value		208.0	124.0	47.0			
Total calorie value ----- 71							

After feeding for one hundred and fifty days the animals were in fine nutritional condition. They were then killed by gas, the kidneys removed at once, fixed in Zenker's solution, embedded in paraffin, sectioned serially, and stained with hematoxylin and eosin. The serial sections were examined carefully and no evidence of kidney pathology found.

CONCLUSION

A high protein diet when fed to either young or adult normal rats for one hundred and fifty days will not produce anatomical lesions in the kidney.

*From the Department of Medicine of Rush Medical College of the University of Chicago and the Presbyterian Hospital in Chicago.
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THE UTILIZATION OF CALCIUM BY RATS*

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DURING recent years the statement that calcium in forms other than that found in milk is not utilizable by the mammalian organism has gained widespread publicity. Of course clinicians who daily treat parathyroid disease, tetany, Paget's disease, osteomalacia, ulcerative colitis, scurvy, rickets, and allergic diseases with inorganic calcium know the fallacy of the statement. Two years ago during a study in our laboratory of the dietary influence on the kidney function in dogs, six animals were fed only raw meat, cod liver oil, sodium chloride, and calcium carbonate for six months. Two females went through full-term pregnancies. At the end of the experiment the long bones were x-rayed and no evidence of calcium absorption could be found. With this information it was thought advisable to feed two groups of rats, one a diet in which the source of calcium was from a supply other than milk and the other a diet in which milk was a large source of calcium. The calcium contents of the two diets were identical. Tables I and II give the diets used.

TABLE I

DIET A

FOOD	AMOUNT IN 100 GM. OF RATION	CARBO- HYDRATE	PROTEIN	FAT	CALCIUM	PHOS- PHORUS	IRON
Whole wheat	38.00	27.4	5.3	0.8	0.017	0.161	0.002
Oats	35.00	23.4	5.6	2.4	0.024	0.139	0.001
Meat powder	15.00	---	13.5	0.9	---	0.083	0.003
Gelatin	5.70	---	5.2	--	---	---	---
Yeast	3.00	1.2	1.4	0.1	0.001	0.042	0.004
Calcium carbonate	1.30	---	---	--	0.520	---	---
Sodium chloride	1.00	---	---	--	---	---	---
Cod liver oil	1.00	---	---	1.0	---	---	---
Ferric citrate	0.01	---	---	--	---	---	0.003
Total	100.00	52.0	31.0	5.2	0.562	0.425	0.013
Caloric value		208.0	124.0	47.0			
Total caloric value	379						

TABLE II

DIET B

FOOD	AMOUNT IN 100 GM. OF RATION	CARBO- HYDRATE	PROTEIN	FAT	CALCIUM	PHOS- PHORUS	IRON
Whole wheat	30.00	21.6	4.2	0.6	0.014	0.127	0.002
Oats	25.00	16.8	4.0	1.8	0.017	0.099	0.001
Meat	10.00	---	9.0	0.1	---	0.055	0.002
Milk	25.00	9.3	6.6	7.1	0.525	0.250	---
Gelatin	5.00	---	4.6	--	---	---	---
Yeast	3.00	1.2	1.4	0.1	0.001	0.042	0.004
Sodium chloride	1.00	---	--	--	---	---	---
Cod liver oil	1.00	---	--	1.0	---	---	---
Ferric citrate	0.01	---	--	--	---	---	0.003
Total	100.00	48.9	29.8	10.7	0.557	0.573	0.012
Caloric value		195.6	119.2	96.3			
Total caloric value	411						

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Ten disease free female rats from the Wistar strain were placed on each diet. The rats were twenty eight days old and were fed for one hundred and fifty days. An excess of food was kept in the cages at all times.

After blood counts were made, the animals were incinerated and the ash analyzed for calcium by the volumetric oxalate method and for phosphorus by the ammonaphthol sulphonic acid method.

RESULTS

At the end of the experiment the blood count and hemoglobin were normal and all the animals appeared healthy.

TABLE III
CALCIUM AVAILABLE IN SOURCES OTHER THAN MILK

RAT	WEIGHT AT END OF EXPERIMENT IN GM	WEIGHT OF ASH IN GM	WEIGHT OF CALCIUM IN GM	PER CENT OF CALCIUM IN ASH	WEIGHT OF PHOSPHORUS IN GM	PER CENT OF PHOSPHORUS IN ASH
A	162	6.271	2.509	40	0.413	7
B	180	7.031	2.883	41	0.400	6
C	196	7.241	2.969	41	0.428	6
D	236	8.969	3.767	42	0.400	4
F	200	7.441	3.125	42	0.434	6
G	228	8.260	3.304	40	0.444	6
H	180	7.301	2.701	37	0.405	6
I	176	7.331	3.152	43	0.428	6
J	Died before experiment was completed					
	Died before experiment was completed					
Average				40		6

TABLE IV
CALCIUM AVAILABLE IN SOURCES INCLUDING MILK

RAT	WEIGHT AT END OF EXPERIMENT	WEIGHT OF ASH IN GM	WEIGHT OF CALCIUM IN GM	PER CENT OF CALCIUM IN ASH	WEIGHT OF PHOSPHORUS IN GM	PER CENT OF PHOSPHORUS IN ASH
AA	196	7.122	2.778	9	0.363	5
BB	190	7.978	2.173	40	0.387	5
CC	196	7.144	2.786	39	0.444	6
DD	184	6.221	2.339	37	0.428	7
EE	188	6.580	2.698	41	0.438	7
FF	204	7.651	3.137	41	0.428	6
GG	206	6.820	2.524	39	0.414	5
HH	188	7.281	3.058	42	0.334	5
II	200	7.230	2.892	40	0.387	5
JJ	168	6.401	2.560	40	0.387	6
Average				40		6

SUMMARY

The calcium and phosphorus content of the ash of rats fed for 150 days on a diet which contained no milk was identical to that of rats which were fed the same diet in which milk was substituted for a part of the food and all of the calcium carbonate.

CONCLUSIONS

From the above data one must conclude that calcium in physical and chemical forms other than that found in milk is utilizable by the mammalian organism.

NORMAL GLUCOSE TOLERANCE TESTS*

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DURING the last five years at the University of Minnesota Students' Health Service any case of glycosuria occurring during an examination was studied further by a glucose tolerance test.† As this investigation progressed, the question was continually raised as to what constituted a normal glucose tolerance test in healthy young adults. In most studies purporting to give "normal" blood sugar values, the figures have been obtained on a large number of patients both bed and ambulatory in type, of different age, weight, and degree of illness. The mean findings have been determined and assumed to be normal. It seemed important then that a series of glucose tolerance tests be done, large enough to be statistically significant on normal people of a given age group under certain standard conditions. The purpose of this communication is to report such a study. In the literature Gray,¹ whose figures are widely accepted, reported that normally the fasting blood sugar value was 80 to 120 mg. per 100 c.e.; that it should not rise above 180 mg. after ingestion of glucose (the average rise, 140 mg.) and that the blood sugar in three hours should be equal to or below that of the fasting period. In the May 15, 1937, issue of the *Journal of the American Medical Association*² in answer to a question on the significance of glycosuria and certain blood sugar values, the following excerpt from the answer is quoted. "A fasting blood sugar of over 120 mg. per hundred c.e. with glycosuria indicates diabetes. The maximum normal blood sugar in a sugar tolerance test (ingestion by an adult of 100 gm. of dextrose) should not exceed 180 mg. at one hour and should drop to 140 mg. at two hours."

Herrman,³ after a thorough review of the literature, concluded that by using any blood sugar method the fasting blood sugar should not exceed 110 mg.; that the rise after ingestion of glucose should not be more than 200 mg.; and that the blood sugar in two hours should be 110 mg. or less.

TABLE I

(In all tables figures expressed as milligrams of sugar per 100 c.e. of blood)

	MEAN FASTING	½ HOUR	1 HOUR	2 HOURS	3 HOURS
Cutaneous blood sugars	95.0	165	137	97.7	79.9
Venous blood sugars	92.2	151	122	85.4	78.4
Difference between cutaneous and venous sugars	2.8	14	15	12.3	1.5

Cavette and Seljeskog⁴ in 1933 reported a comparison of venous and cutaneous sugars obtained during glucose tolerance tests on 21 normal individuals. They had their subjects fast twelve to fourteen hours, after which they obtained a fasting blood sugar and the patient ingested 50 gm. of glucose dissolved in

†A complete analysis of these data will be reported at a later date.

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250 cc. of water, they then obtained blood sugars at intervals of one half hour, one hour, two hours, and three hours after the drink. Table 1 summarizes their results.

Cavette and Seljeskog,⁴ as far as can be determined, had the only report in the literature of glucose tolerance tests done on healthy young normal individuals in which the Gibson⁵ micromethod was used. This same method was used in our investigation. That certain factors may influence the blood sugar value obtained during a glucose tolerance test is well known. Malmros¹ and Sweeney have shown the effect of diet on the glucose tolerance test. Joslin,⁸ Williams and Dick,⁹ Von Noorden and Isaacs¹⁰ have demonstrated that certain diseases, infections or metabolic disturbances may cause a rise in the glucose tolerance curve. Malmros¹ has pointed out the effect of age and Staub¹¹ has drawn attention to the influence of moderate exercise. Our experience in common with that of other observers is that some drugs taken preceding the test may raise blood sugar values. Because the aforementioned factors may affect the results obtained in glucose tolerance tests, it is obvious that they should be eliminated before the results obtained can be interpreted as normal. Hence an individual consulting a physician in his office or an out patient clinic cannot be considered "normal" and should not serve as a subject for the determination of normal blood sugar values during a glucose tolerance test.

Up to 1932 there had been no reports in the literature of blood sugar determinations by the Gibson micromethod during a glucose tolerance test on normal young adults.* This fact prompted the beginning of the investigation, the results of which are reported in this communication.

INTRODUCTION

Any student volunteering as an experimental subject was questioned as to age, family history of diabetes, weight, diet, previous history of glycosuria, drug ingestion, and general health. If the subject gave no history of previous glycosuria or drug ingestion, was eating a normal diet, and, on a complete physical examination, was found to be in good health, he or she was then given a glucose tolerance test.

TECHNIQUE

The subject ate dinner at 6:00 p.m. the evening preceding the test, spent a quiet evening and had nothing by mouth until 8:00 a.m. on the day of the test. At that time fasting venous blood and urine samples were obtained. The patient was then given 100 gm. of glucose dissolved in 250 cc. of water, blood and urine samples were taken one half hour, one hour, two hours and two and one half hours after the drink, and analyzed for glucose.[†] The subject was kept at rest during the test and water intake was restricted to three ounces after each blood sugar.

Two laboratory technicians were employed in this investigation, one doing blood sugar analyses on 38 subjects, and the other analyzing results on 45

*Normal young adult in this instance means that there was no history of glycosuria, presence of disease or a disproportionate amount of carbohydrate, fat or protein in the diet.

†Gibson micromethod for blood sugar. Benedict's qualitative test for urinary sugar.

DATA

Of the total number of normal subjects given glucose tolerance tests (83), 77 were males and 6 were females (Table II). The ages of this group ranged from eighteen to twenty-eight, the average age was 20.45 years. A family history of diabetes was present in 10 subjects. Glycosuria during the glucose tolerance test occurred in nine subjects, only two of whom had a family history of diabetes. There were 49 males in the normal weight group, 20 in the overweight group, and 8 in the underweight group. The average daily diet consisted of: Carbohydrate, 300 to 400 gm.; fat, 90 to 120 gm.; and protein, 60 to 90 gm.

TABLE II
RESULTS OF BLOOD SUGAR DETERMINATIONS ON 83 NORMAL CASES

	FASTING	½ HOUR	1 HOUR	2 HOURS	2½ HOURS
Mean	92.00	134.87	114.64	91.51	83.55
Standard deviation	12.45	24.71	29.30	19.72	18.06

TABLE III
GLUCOSE TOLERANCE TESTS OF 10 NORMAL PERSONS WITH A FAMILY HISTORY OF DIABETES

	FASTING	½ HOUR	1 HOUR	2 HOURS	2½ HOURS
Mean	93.3	143.7	125.6	90.1	74.4

TABLE IV
MEAN BLOOD SUGAR VALUES OF THE NINE INDIVIDUALS WHO SHOWED GLYCOSURIA ON ONE OR MORE OCCASIONS DURING THE GLUCOSE TOLERANCE TEST

	FASTING	½ HOUR	1 HOUR	2 HOURS	2½ HOURS
Mean	96.88	155.66	155.0	106.44	88.55

DISCUSSION

To determine normal blood sugar values for the glucose tolerance test the first requisite is a normal subject, e.g., one free from disease and on a known diet. A second prerequisite is an accurate blood sugar method and competent technicians. The close approximations of the results obtained by the two tech-

TABLE V
COMPARISON OF THE RESULTS OF TWO TECHNICIANS

		FASTING	½ HOUR	1 HOUR	2 HOURS	2½ HOURS
Technician A	Mean	90.64	135.94	111.94	90.50	82.50
	33 cases { Standard deviation	8.91	20.15	23.39	18.20	17.18
Technician B	Mean	92.71	132.76	115.51	90.78	83.29
	45 cases { Standard deviation	14.99	26.25	29.17	18.23	12.03

nicians doing this study and the consistent results one may obtain by the Gibson method are demonstrated in Table V. The mean blood sugar values obtained in this investigation are below those generally accepted as normal in the literature.

This study was started in the hope that the findings would enable one to predict whether or not any single glucose tolerance test was abnormal or varying within normal limits. When the mean values and variation from the means

had been determined statistically for the normal group their tolerance tests as well as all initial tolerance tests being done routinely on subjects with glycosuria (this study mentioned in opening paragraph) were placed in one of the five groups listed below

Group I in which the highest blood sugar after ingestion of glucose was from two to three or more standard deviations from the calculated normal mean (Table II)

Group II in which at least two of the blood sugars during the test were two to three or more standard deviations above the corresponding mean of the normal series

Group III in which at least three of the blood sugar values were two to three or more standard deviations above the corresponding mean of the normal series

Group IV in which at least four of the blood sugar values were two to three or more standard deviations above the corresponding mean of the normal series

Group V in which all five blood sugars were two to three or more standard deviations above the normal mean of the series

The normal group and some hundred and fifty initial glucose tolerance tests were so classified and were studied further by means of other glucose tolerance tests at intervals of six to twelve months over a period of two to four years. One could assume that a subject with a tolerance test classed in Group I would have less chance of developing a further disturbance of tolerance than an individual with a Group III curve and conversely an individual with a Group V test would have a much less chance of recovering a normal test than one in Group II or III, etc. This assumption in the main was found to be correct but not in a large enough number of cases so that the group classification could be used accurately to determine whether or not any given test was normal and that the individual with such a test need pay no further attention to it

Numerous statistical formulas were then applied to the initial curve to determine whether or not they could accurately predict the then known outcome of any given case. Dr. Tietloar,¹⁷ Assistant Professor of Biometry at the University of Minnesota, was one who suggested certain formulas that might be used. It should be noted that the behavior of each succeeding tolerance test had already been determined during the years this study was being carried out so that there was a definite check on the efficacy of each formula tried. It was found the more complex the formula the less liable it was to predict the correct outcome. From this statistical study, however, I discovered one formula which accurately predicted whether or not one was dealing with a normal tolerance test in about ninety to ninety five per cent of the cases studied. The formula is as follows $\lambda = \text{fasting} + \text{highest} + 2\frac{1}{2} \text{ hour blood sugar values expressed in mg per hundred c.c. of blood divided by three}$. In interpreting the results if λ is above 140 the glucose tolerance test should be classed as abnormal until proved otherwise by further tests and clinical observations. In border line cases just above or below 140 one should study further any individual case before a final conclusion is drawn. If the figure is above 145 to 150, one is almost certain to be dealing with a disturbance in glucose tolerance. Since the original observations on one hundred and fifty tolerance tests were done the formula has continued to aid the writer in his interpretation of over five hundred tests, thus its depend-

ability seems established. It should be remembered, however, that any formula may fail in any single instance.

Other incidental though interesting findings in this study are the high incidence 10.8 per cent (9 of 83) of glycosuria occurring during a tolerance test on healthy young adults (Table IV). The fact that normal people with family history of diabetes do not have evidence of a disturbed glucose tolerance test has been frequently observed.

TABLE VI

RESULTS OF GLUCOSE TOLERANCE TESTS ON MALE SUBJECTS, MALE SUBJECTS IN NORMAL, OVER- AND UNDERWEIGHT GROUPS*

		FASTING	½ HOUR	1 HOUR	2 HOURS	2½ HOURS
Normal weight 49 cases	Mean	91.17	135.61	114.38	88.67	82.96
	Standard deviation	12.27	25.90	27.51	19.24	19.89
Overweight 20 cases	Mean	94.50	135.50	122.00	95.50	83.50
	Standard deviation	9.90	25.19	34.65	19.61	12.20
Underweight 8 cases	Mean	91.50	122.75	98.63	93.50	81.63
	Standard deviation	17.99	15.70	23.15	16.10	17.68

*Normal weight=90 to 110 per cent of normal weight as calculated from standard insurance tables.

Overweight=Over 110 per cent of normal weight as calculated from standard insurance tables.

Underweight=under 90 per cent of normal weight as calculated from standard insurance tables.

Comparing the normal, over- and underweight groups (Table VI) one sees that in the first two groups the half-hour blood sugar values are the same while in the underweight group it is lower. No conclusions can be drawn from these findings as the series is too small. It is interesting, however, that the overweight group's blood sugar values are practically the same as the normal weight group's.

CONCLUSIONS

1. Eighty-three subjects between the ages of eighteen and twenty-eight, in good health and on a diet of 300 to 400 gm. of carbohydrates, 90 to 120 gm. of fat, and 60 to 90 gm. of proteins, were given a glucose tolerance test. The mean blood sugar values (expressed in mg. per 100 c.c. blood) were: Fasting, 92; ½ hour, 134.8; 1 hour, 114.64; 2 hours, 91.51; 2½ hours, 83.55.

2. The figures are below those generally accepted as normal.

3. Statistical formulas cannot be applied to a single glucose tolerance test to predict accurately the ultimate clinical prognosis of a patient with a disturbed carbohydrate metabolism. The formula:

$$x = \text{fasting} + \text{highest} + 2\frac{1}{2} \text{ hr. blood sugar values expressed in mg./100 c.c. blood}$$

3

is more accurate than any other statistical formula that was tried. If x exceeds 140, the patient should be observed for evidence of further disturbance of the glucose tolerance test.

4. The Gibson micromethod of blood sugar determination is a satisfactory method.

5 One finds on doing glucose determinations on urines collected during the glucose tolerance test that glycosuria is not uncommon, e g, 9 cases or approximately 11 per cent of the group showed glycosuria during the test

6 Although the overweight group is small (20 cases), it seems that in young adults obesity does not predispose the individual to a disturbed carbohydrate metabolism

7 In our series subjects with family history of diabetes showed no evidence of diminished carbohydrate metabolism as determined by the glucose tolerance test

NOTE Acknowledgment is here given to Dr H S Diell, Dean of the University of Minnesota Medical School, for his suggestions regarding the statistical approach to this problem Our appreciation to Mrs Gladys Benson Hansen, Miss Miriam Lipser our blood chemists, Misses Grace Williams, Mary Ryan who obtained the blood samples and Miss Martha Lavell who did many of the various statistical analyses during this study

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HYPERPROTEINEMIA IN MULTIPLE MYELOMA*

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HYPERPROTEINEMIA as a manifestation of multiple myeloma has been reported so frequently in the past few years that it is being considered as one of the important points in the differential diagnosis of this condition This association was first emphasized by Peilzweig, Delrue and Geschickter¹ in 1928 although Jacobson² in 1917 found 7.86 per cent of a substance he believed to be Bence Jones protein in blood obtained from the heart of a patient who had died of multiple myeloma and Pribam³ in 1925, reported a case with a total protein of 87 per cent We have been able to find 52 cases of multiple myeloma with hyperproteinemia almost all of which have been reported since 1928 (Table I)

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The normal values for plasma proteins range from 6 to 8.7 per cent,^{7, 15 35-37} so that a total plasma protein of 9 per cent or over may be regarded as a definite increase. We have included in Table I only those cases in which the protein content of the plasma reached or exceeded this figure. The highest total protein reported was 18.37 per cent (Case 32). In those cases in which a protein partition was done the albumin fraction was usually found to be within normal limits but the globulin fraction was consistently increased. The fibrinogen varied from normal to very high levels. Complete absence of serum albumin was found in one case (Case 38), but the patient's blood fibrinogen was 8.42 per cent. Although extremely high globulin values were occasionally found, there is no agreement as to which globulin fraction is responsible for this increase. It has been shown that the accurate differentiation of euglobulin and pseudoglobulin I and II is difficult³⁸ and that the results may vary with a difference in the dilution of the serum³⁹ and with different techniques.³⁹ In the reported cases the euglobulin was usually greater than the pseudoglobulin, although the reverse was true in Cases 36 and 39. The hyperproteinemia apparently is not due to the presence of Bence-Jones protein, for most investigators have not been able to detect its presence. However, Jacobson² found 7.86 per cent of Bence-Jones protein in blood removed postmortem and Cantarow²⁶ found 5.2 per cent in a case (Case 40) with a total protein of 11.3 per cent. Shirer, Duncan, and Haden⁸ apparently demonstrated Bence-Jones protein in large amounts in their two cases. Gabbe⁴⁰ detected 0.42 per cent of this protein in the blood of a patient who showed 30 per cent Bence-Jones protein in the urine and small amounts have been found in the blood by other investigators.⁴¹⁻⁴⁵ It has been suggested that Bence-Jones protein is easily excreted by the kidney due to its relatively small molecular weight and therefore does not accumulate in the blood.²⁹ Bence-Jones proteinuria was sought for in 35 of the reported cases and found in 23 of them.

The general incidence of hyperproteinemia in multiple myeloma cannot be ascertained accurately from Table I for these patients were deliberately selected for report because they showed hyperproteinemia. The blood protein may reach or exceed 9 per cent not only in multiple myeloma, but also in kala-azar,⁴⁶ syphilis,⁴⁶ myelogenous leucemia,²⁵ "a toxic infectious lesion of the central nervous system,"⁴⁷ lymphogranuloma inguinale,³⁴ large kidney tumors,⁴⁸ sarcoid of Boeck,⁴⁹ and in lymphosarcoma.³⁴ In Rowe's case of inguinal lymphadenopathy⁵⁰ the total protein was found to be approximately normal but rose to 10.4 per cent after venous stasis had been produced in the arm.

The blood protein studies which we made on 10 patients with multiple myeloma are summarized in Table II. It will be noted that hyperproteinemia (total protein over 9 per cent) occurred in 3, or 30 per cent of the 10 cases. In 3 additional cases (Cases 5, 6, and 7) the total protein was over 8 per cent, but these values must be considered within the upper limits of normal. In the cases with a distinct hyperproteinemia, the albumin fraction was either normal or slightly below and the increase in the total protein was due chiefly to globulin. The fibrinogen was increased in Cases 2, 7, and 8 but was approximately normal in the others. In all but the first 3 cases there was an alteration in the normal albumin-globulin ratio, with actual inversion of the ratio in four instances (Cases

TABLE I
BLOOD PROTEIN STUDIES FROM THE LITERATURE

AUTHOR	YEAR	PLASMA OF SERUM	TOTAL PROTEIN GM PER CENT	ALBUMIN GM PER CENT	GLOBULIN GM PER CENT	ELGLOBULIN GM PER CENT	PSUEDO GLOBULIN GM PER CENT	PER CENT I AND II	FIBRINOGEN GM PER CENT	BENCE JONES PROTEIN IN URINE
1 Jacobson ^a	1917		7.56*							+
2 Perlzweig ¹	1928		13.81	4.06	9.09	6.79	0.63	1.49	0.69	+
3 Bannick ⁴	1929	Serum	9.00	2.50	6.50					+
4 Bannick	1929	Serum	10.54	1.45	6.09	4.40	1.14	0.55		+
5 Johansen ⁵	1931	Serum	11.00	0.36	0.48	5.83	0.97	1.69	0.92	0
6 Jores ⁶	1931	Blood	9.32	3.32	5.81				0.19	+
7 Kumpf	1931	Plasma	14.13	4.65	9.48				1.45	0
8 Shurer ⁸	1932	Serum	13.78	2.14	11.74					0
9 Shurer	1932	Serum	10.02	2.30	7.72	6.32	0.66	0.74		+
10 Reimann ⁹	1932	Plasma	10.12	0.90	9.10	2.37	1.47†		5.48	0
11 Medes ¹⁰	1932		16.00							-
12 Bennhold ¹¹	1932	Serum	11.40	1.80	9.60					-
13 Bennhold	1932	Serum	9.50	2.60	6.90					-
14 Bennhold	1932	Serum	16.00	1.20	14.80					-
15 Veil ¹²	1932	Serum	14.00	6.20	7.90					-
16 Magnus Levy ¹³	1933	Serum	9.90	3.30	6.60	4.10	2.50†		0.50	+
17 Magnus Levy	1933	Serum	13.00	3.50	9.50	7.30	2.20†		0.50	+
18 Magnus Levy	1933	Serum	12.00	4.70	7.30	6.10	1.20†			+
19 Veil ¹⁴	1933	Serum	12.10	5.70	7.0					+
20 Peters ¹⁵	1933	Serum	High							-
21 Peters	1933	Serum	High							-
22 Bonninger ¹⁶	1933	Blood	13.00							-
23 Wintrobe ¹	1933	Plasma	11.90		Increase†					0
24 Herbert ¹⁸	1933		12.6							-
25 Johansen ¹⁹	1934	Plasma	10.80	2.08	8.72					0
26 Johansen	1934	Plasma	11.00	0.36	8.48	5.83	96	1.68	0.92	0
27 Johansen	1934	Plasma	13.50	1.96	10.15	8.03	33	68	1.32	+
28 Johansen	1934	Plasma	11.39	1.96	9.14	7.25	1.45	24	0.28	0
29 Foord ²⁰	1935	Plasma	13.20	4.30	8.30				0.60	0
30 Foord	1935	Plasma	15.00	5.50	9.80					+
31 Foord	1935	Plasma	11.37	1.86	9.51					-
32 Foord ²¹	1935	Plasma	18.77	6.00	11.90				0.47	+
33 Foord	1935	Plasma	12.74	2.87	9.38				0.49	+
34 Sweigert ²²	1935	Plasma	12.00	1.80	10.20				0.75	+
35 Bruer ²³	1935	Plasma	11.66	2.94	8.72	4.41	4.31†		0.31	0
36 Jacobson ²⁴	1935	Serum	10.50	1.60	8.90	3.10	5.80†			-
37 Jacobson	1935	Serum	11.10	2.20	8.90	7.90	1.00†			-
38 Jacobson	1935	Serum	12.70	0.00	12.70	10.00	2.70†			-
39 Gros ²⁵	1935	Serum	11.68	2.54	9.14	12	5.34	3.68	0.28	0
40 Cantarow ²⁶	1935	Serum	11.30	2.80	8.50					+
41 Cabot Case ²⁷	1935	Serum	11.70							+
42 Robbins ²⁸	1935	Serum	13.10	2.80	10.30					0
43 Berglund ²⁹	1935	Plasma	16.10	2.50		7.41	0.59	0.38	5.22	+
44 Berglund	1935	Plasma	17.58	1.16		4.58	2.50	0.51	8.42	+
45 Geschlechter ³⁰	1936	Plasma	11.07							-
46 Cabot Case ³¹	1936	Serum	11.40							+
47 Gutman ³²	1936	Serum	9.00							+
48 Gutman	1936	Serum	9.90							+
49 Bing ³³	1936	Serum	11.02	2.86	8.16					0
50 Bing	1936	Serum	12.69	3.07	9.62					+
51 Gutman ³⁴	1936	Serum	10.09	3.5	7.4	4.6				-
52 Gutman	1936	Serum	9.9	2.9	7.0					-

*Reported as Bence Jones protein total protein not determined

†Total for I and II

- Information not available

TABLE II
CHEMICAL STUDIES IN 10 CASES OF MULTIPLE MYELOMA

AGE	SEX	STAGE OF DIS- EASE WHEN THE STUDIES WERE MADE	SERUM OR PLASMA	TOTAL PROTEIN GM. %	ALBUMIN GLOBULIN		FIBRIN- OGEN GM. %	BENCE- JONES BODIES IN URINE	SERUM CALCIUM MG. %	SERUM PHOS- PHORUS MG. %	RENAL FUNCTION	CELL TYPE	
					GM. %	GM. %							
1	48	M	4 mo. after onset of symptoms	P	6.85	3.65	2.76	0.44	0	10.20	3.20	Blood nitrogen normal	-
2	49	M	1st year of a 6- year course	P	7.38	4.61	1.85	0.92	+	12.80	4.10	-	-
3	45	F	3rd year of a 4- year course	S P P	7.00 7.17 6.37	5.40 3.38 3.59	1.60 3.32 2.18	- 0.47 0.60	0	10.70	3.40	Blood nitrogen normal Urea clearance 116% of normal Maximum specific gravity of urine 1.024	Biopsy of scapula, normal plasma cell type
4	51	M	1 year after onset; 1 mo. before death	P	7.53	3.71	3.21	0.61	+	15.00	5.00	-	-
5	57	M	1 mo. before death; 1 year from onset	P P P	6.85 8.05 8.00	3.21 3.96 2.60	3.77 3.54 4.97	0.56 0.55 0.43	+	9.75	2.86	Blood urea nitrogen 25.9 mg. % Urea clearance 58% of normal Maximum specific gravity of urine 1.034	Biopsy of bone, normal plasma cell type

TABLE II—CONT'D

6	57	M	1 mo before death, 3 years from onset	P	837	253	116	0.68	+	1310	286	Blood nitrogen normal Urea clearance 60% of normal Maximum specific gravity of urine 1.011	Autopsy, plasma cell type
7	28	F	2 days before death, 6(?) years from onset	P P P	830 800 880	170 180 376	160 220 330	1.20 1.00 —	0	1500	371	Blood nitrogen normal Urea clearance 10% of normal Maximum specific gravity of urine 1.017	Autopsy, plasma cell type
8	66	M	Pt bedridden 2 mo later, 3 years from onset	P S	904 915	301 268	478 647	1.15 —	?	1112	1.0	Blood urea nitrogen 21.0 mg % Blood creatinine 1.8 mg % Blood urea acid 51 mg %	—
9	52	F	2 years from onset of symptoms	S S P S	1230 1030 913 1240	150 110 200 210	1050 800 661 1010	— — 0.52 0.20	+	171 167	268 250	Blood nitrogen normal Urea clearance 26% of normal Maximum specific gravity of urine 1.01	Biopsy of skull, hist. cell type
10	54	F	10 days before death, 6 mo from onset	P P	1000 1159	323 305	639 809	0.78 0.15	0	106	1.3	Blood creatinine 10.2 mg % Blood urea acid 5.4 mg % Blood urea nitrogen 61.0 mg %	Autopsy, plasma cell type

Blood protein determinations were done by Howe's method. J Biol Chem 49: 93 and 109, 1921 and by the colorimetric method of Anker h and Gibson with a corrected factor. J Lab & Clin Med 18: 816, 1933

6, 8, 9, and 10). The blood was not examined for Bence-Jones protein, and no attempt was made to fractionate the globulin. Examination of the urine revealed Bence-Jones protein in five cases.

Hypoproteinemia has been reported in multiple myeloma by Chester.⁵¹ In two cases he found a total serum protein of 3.58 per cent and 4.74 per cent, respectively. He believed that all cases of multiple myeloma would eventually show these low values if the studies were carried out late in the course of the disease and in support of this he cited the case of Thannhauser and Krauss⁵² in which the blood protein fell from 6.12 per cent to 4.49 per cent in four months. Our observations do not substantiate this idea because, although the majority of the determinations were made in an advanced stage of the disease, they gave normal, or high, values. In Case 7, with a proteinemia of 8.86 per cent, the determinations were made two days before death, and in Case 10 the determinations made ten days before death showed 11.59 per cent total protein. In Cases 4, 5,

TABLE III

BLOOD CHEMICAL STUDIES IN VARIOUS CONDITIONS INVOLVING THE SKELETAL SYSTEM

	AGE	SEX	DIAGNOSIS	TOTAL PROTEIN GM. %	ALBUMIN GM. %	GLOBULIN GM. %	FIBRINOGEN GM. %	SERUM CALCIUM MG. %	SERUM PHOS- PHORUS MG. %
1	41	M	Carcinoma meta- static from pros- tate	4.81	2.32	1.98	0.50	12.50	4.00
2	63	M	Carcinoma meta- static from pros- tate	8.00	3.90	4.10	—	10.80	3.40
3	55	F	Carcinoma meta- static from breast	5.62	2.14	3.36	0.12	10.00	3.70
4	45	F	Carcinoma meta- static from breast	6.05	2.69	2.89	0.50	9.50	4.80
5	58	F	Carcinoma meta- static from breast?	6.06	4.17	1.84	—	10.10	3.50
6	30	M	Carcinoma meta- static from stomach	6.30	—	—	—	18.50	4.60
7	14	M	Ewing's tumor	8.8 7.30	— 2.80	— 4.20	— 0.30	12.80	1.94
8	18	M	Ewing's tumor	6.35	3.00	2.78	0.57	19.30	3.60
9	4	M	Myeloblastoma	5.77	2.80	2.68	0.28	9.20	4.40
10	52	F	Tumor, metastatic from brain, type?	7.11	3.68	2.96	0.47	12.20	3.30
11	27	M	Myeloid leucemia	7.30	3.50	3.40	0.40	10.10	3.70
12	43	F	Acromegaly	6.50	2.60	3.90	—	10.80	3.94
13	55	M	Acromegaly	6.90	2.54	3.36	—	10.40	3.90
14	68	F	Senile osteoporosis	7.70	3.10	4.60	—	9.85 13.60	3.64 —

and 6 the total protein was normal one month before death. Although we cannot be sure that a drop in the blood protein had not occurred, it is evident that hypoproteinemia was not present.

There are several phenomena which may be encountered in the routine examination of patients that suggest the presence of hyperproteinemia. Unusual rouleaux formation or actual autohemagglutination occurring on wet or dry blood smears was noted by Reimann⁹ and by Foord.²⁰ Difficulty or inability to count the erythrocytes because of the formation of a granular precipitate when the blood was mixed with Hayem's solution was observed by Reimann,⁹ Bön-

niger,¹⁶ Jacobson,¹⁴ and Foord.²⁰ The appearance of a heavy milky precipitate when blood serum was heated preparatory to doing a Wassermann test was noted by several investigators.⁸⁻¹³⁻¹⁴ Unusually rapid coagulation of the blood was reported by Johansen.²⁰ In Case 10 of our series there was marked rouleaux formation on fixed blood smears but no difficulty was encountered in doing either the erythrocyte count or the Wassermann test.

Table III gives the results of blood chemical studies in 14 cases with other types of skeletal lesions. Hyperproteinemia (9 per cent or more) did not occur, although the total protein reached 8.8 per cent in one case of Ewing's tumor. Absolute hyperglobulinemia was not found, but the albumin globulin ratio was reversed in 7 cases. The fibrinogen values were all within normal limits. The fact that clearly defined hyperproteinemia and hyperglobulinemia seem to be limited to multiple myeloma may be useful in the differential diagnosis of multiple lesions involving the skeletal system.

Serum calcium and phosphorus determinations in the 10 cases of multiple myeloma are shown in Table II. It will be noted that in 5 of them there was an increase of the serum calcium to 12 per cent or over, and that in 3 of these 5 cases there was an associated increase in the inorganic phosphorus. In the other 7 cases the phosphorus values were either normal or slightly above. These observations are in agreement with those of Gutman and others²¹ who recently reported 4 cases of multiple myeloma with an increased serum calcium but with normal or slightly elevated phosphorus values. In an extensive review of the literature they collected 36 additional cases presenting similar features. Reference to Table III, however, shows that this is not limited to multiple myeloma, for marked hypercalcemia was present in one case of Ewing's tumor (19.30 per cent) and in a case of carcinoma metastatic from the stomach (18.50 per cent). In neither of these was the serum phosphorus greatly altered. These observations are of value in differentiating multiple myeloma or other malignancies involving the skeletal system from hyperparathyroidism in which there is, as a rule, an increased blood calcium but a lowered phosphorus content.²²

Renal function studies or blood nitrogen estimations were made in 8 of the 10 cases in our series (Table II). Decreased ability of the kidney to concentrate the urine was present in Cases 6 and 9 and a lowered urea clearance was found in Cases 5, 6, 7, and 9. One patient (Case 10) presented all the manifestations of renal insufficiency and died in uremia with a creatinine of 10.2 and a urea nitrogen of 64 mg per cent. The blood pressure was normal and there was no clinical evidence of arteriosclerosis. Repeated examination of the urine showed a persistent albuminuria but Benedict Jones protein was never detected. Histologic examination of this patient's kidneys revealed only a minor degree of arterial thickening. A majority of the glomeruli showed no appreciable alteration and only a very few were hyalinized. There was an extensive increase in the interstitial fibrous tissue and a very slight amount of chronic inflammatory reaction. The most striking alterations occurred in the tubules. Many of these contained dense hyaline casts, some of which were surrounded by zones of loose granular hyaline debris, but no material of a crystalline nature was found in the casts. A few giant cells of the foreign body type were located adjacent to the casts and within the lumina of the tubules. Other casts were surrounded by zones of desquamated tubular epithelium and clusters of neutrophils. Some tubules were

dilated but contained no casts whereas others were constricted and atrophic. Beneath the pelvic epithelium there were lymphocytes, plasma cells and a few eosinophiles. It is possible that some of the kidney scarring was the result of an ascending infection but it seemed probable that the significant changes were associated with the casts in the tubules.

Hamman⁵³ stated that renal insufficiency with nitrogen retention but without an elevated blood pressure was characteristic of multiple myeloma, and that under these circumstances a careful roentgenologic study of the skeletal system should always be made. Geschieker and Copeland³⁰ estimate that renal lesions are present in 70 per cent of the cases of multiple myeloma. Although the blood pressure was usually normal, hypertension occurred occasionally. They

TABLE IV
BLOOD STUDIES IN 10 CASES OF MULTIPLE MYELOMA*

	HEMOGLOBIN (SAILI) PER CENT OF NORMAL	ERYTHROCYTES, MILLIONS	LEUKOCYTES	NEUTROPHILES	EOSINOPHILES	BASOPHILES	LYMPHOCYTES	MONOCYTES	DEGENERATED CELLS	PATHOLOGICAL LYMPHOCYTES	MYELOCYTES	NORMOBLASTS	UNCLASSIFIED CELLS
1	85	3.53	5,200	65	1	1	26		7				
2	87	3.91	3,050	56	2		24	5	1				
3	94	4.76	8,800	55			12	3	3	12			
4	35	1.59	11,100	65			22		2	37			
5	47	1.95	3,300	56			41	3			12	2	3
6	64	2.72	6,900										
7	78	3.54	8,400	79			16	5					
8	87	3.80	7,800	67	3		26	4					
9	39	2.26	4,500	48	1		40	3	4		4		
10	22	1.19	2,850	44			56						

*These cases are in the same order as in Table II.

found also that the kidney was smooth and white and on microscopic examination showed pronounced tubular degeneration. They attributed contraction of the kidney and glomerular changes to a secondary sclerotic process. Bell⁵⁴ in 1933 described the kidney changes in eleven cases of multiple myeloma and thoroughly reviewed the literature on this phase of the subject. He found that renal insufficiency was a frequent complication in the advanced stages of multiple myeloma. In his opinion, the only direct effect of multiple myeloma on the kidney is the formation of casts composed of Bence-Jones protein which obstruct the tubules and cause them to become atrophic. Obstruction of large numbers of the tubules leads to extensive atrophy of the cortex and, eventually, to renal insufficiency. The renal changes which we found in our case were the same as those described by Bell⁵⁴ in so far as the histological alterations are concerned, but it is doubtful if the tubular casts were composed of Bence-Jones protein, for we were unable to demonstrate the presence of this protein in the urine in spite of repeated attempts to do so.

The hematologic features of multiple myeloma are not striking and are in no way diagnostic (Table IV). The severity of the anemia will depend upon the amount of bone marrow involvement and the duration of the disease. The hemoglobin and erythrocytes are diminished about equally with the result that the color index approaches unity. In our cases the total leucocyte count varied

from 2,850 to 11,100, and there was no discernible relationship between the leucopenia and the degree of reduction in the number of the erythrocytes. Two patients (Cases 4 and 9) showed a few myelocytes in the peripheral blood and in Case 4 nucleated erythrocytes were present. Although a majority of the cases presented an essentially normal differential leucocyte count it must be realized that abnormal cells appear occasionally.

SUMMARY AND CONCLUSIONS

1 Hyperproteinemia was found in 3 of our 10 cases of multiple myeloma and in addition we have collected 52 cases from the literature

2 The fact that hyperproteinemia and hyperglobulinemia are practically limited to multiple myeloma may be useful in the differential diagnosis of multiple lesions involving the skeletal system.

3 Hypercalcemia together with a normal or slightly increased inorganic serum phosphorus is frequently found in multiple myeloma, but may occur with other bone lesions.

We are indebted to Doctor R. E. Shaw for the pathologic observations upon the kidney.

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AN UNUSUAL BACILLUS FROM A CASE OF BACTERIAL ENDOCARDITIS*

GUSTAV FRIEDMAN, M.D., CHICAGO, ILL.

A PREVIOUSLY undescribed bacillus was obtained on three different occasions from the blood stream of a patient with bacterial endocarditis. Its source and portal of entry are not known.

CASE HISTORY

The patient was a thirty-five year old woman when first seen in the clinic. At the age of thirteen years she had tonsillitis and sore throat. These were followed by an attack of swelling, tenderness and redness of several joints. Asbestos and edema appeared after the acute attack of rheumatism. She was told she had heart disease. At the age of sixteen her tonsils were removed. She married at twenty-four years of age and went through two pregnancies, at twenty-six and thirty years, without difficulty. The patient gained 15 pounds during the latter pregnancy and then voluntarily reduced her weight to 104 pounds. She never felt strong again and began to have mild dyspnea on exertion. During the five months prior to her admission to Billings Hospital she had several bouts of perspiration associated with sensations of chilliness. She had a cough that was productive of mucopurulent sputum for three weeks just before hospitalization. Although the patient was in bed she experienced dull aching pain over the precordium for a few days before admission. She also had pain in the right costovertebral angle and described her urine as being "unusually dark." Several weeks before she was first seen a finger tip was tender and swollen.

The admission examination revealed a sick looking, undernourished, dyspneic woman whose temperature was 100.4° F. The pulse rate was 108/min, the respiratory rate was 20/min and the blood pressure was 112/50. The diastolic range was poorly defined and may have been lower. Other salient features of the examination included scattered petechiae in the skin, a large, hyperactive heart and a large firm palpable spleen. The precordium heaved synchronously with the heart beat. A systolic thrill was palpable over the apex of the heart. The apical thrust was diffuse and prominent. The second sound was not heard at the aortic region. A loud systolic murmur was audible over the entire precordium. A short, faint diastolic murmur was detected over the aortic region replacing the second sound. The pharynx was not inflamed.

The laboratory procedures gave the following results. On May 8, May 14, and August 5, 1936 blood cultures were taken. On each occasion a bacillus was obtained in pure culture. Agglutinations with the patient's serum against the typhoid, paratyphoid and *B. abortus* groups were positive only with the *E. typhosus* antigen in a dilution of 1/20. The others were entirely negative.

Stereoscopic x-rays of the chest showed the heart to be "slightly less than 65 per cent over size by film tracing." Early congestive changes were reported in both lungs.

Besides a tachycardia of 100/min an electrocardiogram showed broad P waves in all leads and T₂ to be low in amplitude.

The blood Kahn was weakly positive and the Wassermann was negative.

From May 8 to May 15, 1936, while the patient was in the hospital, the leucocyte count varied from 9,100 to 11,100 cell/cmm. The differential count showed a mild absolute increase in polymorphonuclear leucocytes. A moderate secondary anemia was present at all times.

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GUSTAV FREEMAN, MD, Chicago, Ill

A PREVIOUSLY undescribed bacillus was obtained on three different occasions from the blood stream of a patient with bacterial endocarditis. Its source and portal of entry are not known.

CASE HISTORY

The patient was a thirty five year old woman when first seen in the clinic. At the age of thirteen years she had tonsillitis and sore throats. These were followed by an attack of swelling, tenderness, and redness of several joints. Ascites and edema appeared after the acute attack of rheumatism. She was told she had heart disease. At the age of sixteen her tonsils were removed. She married at twenty four years of age and went through two pregnancies, at twenty six and thirty years, without difficulty. The patient gained 15 pounds during the latter pregnancy and then voluntarily reduced her weight to 104 pounds. She never felt strong again and began to have mild dyspnea on exertion. During the five months prior to her admission to Billings Hospital she had several bouts of perspiration associated with sensations of chilliness. She had a cough that was productive of mucopurulent sputum for three weeks just before hospitalization. Although the patient was in bed she experienced dull aching pain over the precordium for a few days before admission. She also had pain in the right costovertebral angle and described her urine as being "unusually dark." Several weeks before she was first seen a finger tip was tender and swollen.

The admission examination revealed a sick looking, undernourished, dyspneic woman whose temperature was 100.4° F. The pulse rate was 108/min, the respiratory rate was 20/min and the blood pressure was 112/50. The diastolic range was poorly defined and may have been lower. Other salient features of the examination included scattered petechiae in the skin, a large, hyperactive heart and a large firm palpable spleen. The precordium heaved synchronously with the heart beat. A systolic thrill was palpable over the apex of the heart. The apical thrust was diffuse and prominent. The second sound was not heard at the aortic region. A loud systolic murmur was audible over the entire precordium. A short, faint diastolic murmur was detected over the aortic region replacing the second sound. The pharynx was not inflamed.

The laboratory procedures gave the following results. On May 8, May 14, and August 5, 1936 blood cultures were taken. On each occasion a bacillus was obtained in pure culture. Agglutinations with the patient's serum against the typhoid, paratyphoid and *B. abortus* groups were positive only with the *E. typhosus* antigen in a dilution of 1/20. The others were entirely negative.

Storeoscopic x rays of the chest showed the heart to be "slightly less than 65 per cent over size by film tracing." Early congestive changes were reported in both lungs.

Besides a tachycardia of 100/min an electrocardiogram showed broad P waves in all leads and T₂ to be low in amplitude.

The blood Kahn was weakly positive and the Wassermann was negative.

From May 8 to May 15, 1936, while the patient was in the hospital, the leucocyte count varied from 9,100 to 11,100 cells/cmm. The differential count showed a mild absolute increase in polymorphonuclear leucocytes. A moderate secondary anemia was present at all times.

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The urinary findings were usually not abnormal. On one occasion a trace of albumin was found. Rarely red and white cells appeared in the sediment.

The patient was last seen in August of 1936, three months after the first two blood cultures were obtained. The third culture was taken at that time. She died about a month later at home. An autopsy was not obtained.

It is apparent that this is a case of bacterial endocarditis in a person who had rheumatic fever in childhood. The myocardium was strong enough to carry through two pregnancies without incident. From the story one can judge the duration of the bacterial endocarditis to have been at least nine months. Most likely the mitral and aortic valves were ulcerated.

Description of Organism.—The bacillus obtained from this case grew out in pure culture on all three attempts. The first and the last were three months apart. No other organisms or pleomorphic variants were discovered. The organism was a gram-negative bacillus with pointed ends. The bacilli had an average length of 2μ and a thickness of 0.28μ . They varied slightly in length and very little in width. Stained by the Gram method barring was some-

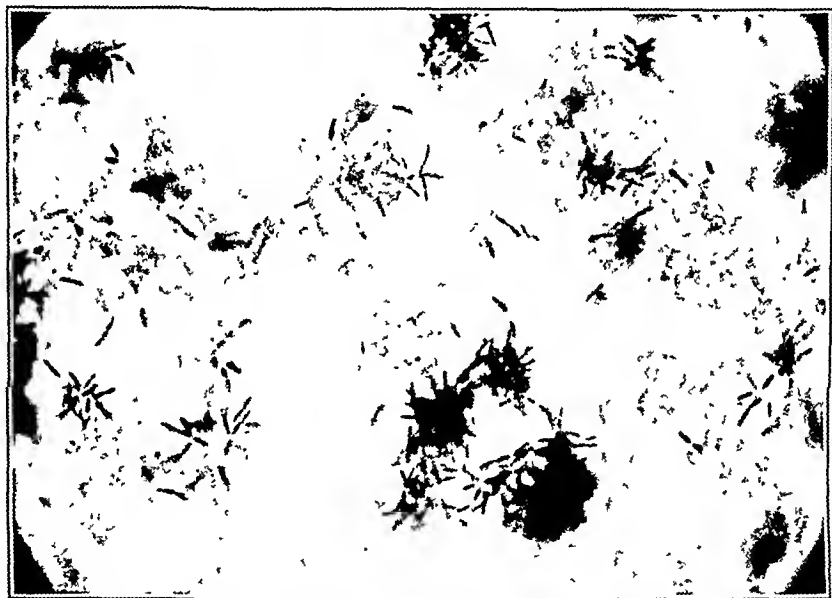


Fig. 1.—A smear from a broth culture. Stained by the Gram method. Magnification $\times 2300$.

times seen; also bipolar forms appeared in the same smear. The fresh twenty-four hour cultures taken from the patient tended to be slightly gram-positive but gentian violet was not retained in cultures older than twenty-four hours. Subcultures were invariably gram-negative. The bacilli tended to form short filaments which had in places gram-positive spore-like ends. Occasionally an isolated gram-positive organism appeared in a smear in the form of a lancet-shaped diplococcus. The bacillus was not acid fast. In dextrose broth it grew as a granular deposit. It grew slowly at first on beef infusion agar but readily after several subcultures on that media. The colonies were smooth and round. They resembled diphtheria colonies in size, shape, and color. On chocolate agar, they were discrete yellowish colonies similar to those grown on beef infusion agar except for the slight increase in pigmentation. Although subcultures were not at first successful on litmus lactose and sheep blood, beef extract media, the organisms grew out after several trials. The organisms growing in broth were not motile. No indol was formed. Gelatin was not liquefied. The following sugars were fermented with production of acid and gas: dextrose, mannite, maltose, and sorbitol. These were not affected: lactose, sucrase, and galactose. Acid without gas was formed in trehalose. No other sugars were used.

The organism was agglutinated by the patient's serum in a dilution of 1:5120. In three normal controls, slight, poorly defined agglutination was noted. The organism was not pathogenic for mice, rabbits, or guinea pigs.

The organism ceased to grow after several subcultures and was lost. From the above findings it is likely that this organism falls into the order Actinomycetales. It does not parallel any other particular species.

DISCUSSION

At least 26 different species of bacteria have been isolated in pure culture from the blood streams of patients with endocarditis. The disease has been described in association with virus disease.¹ The usual habitats of these organisms include all recognized portals of entry of the body. It is not surprising to encounter an unusual invader in the blood stream of individuals with bacterial endocarditis.

Reports have appeared in which diphtheroids were grown from the blood of patients with bacterial endocarditis and green forming streptococci were recovered from the vegetations in these individuals after death.² Cases and experiments have been described in which diphtheroid organisms obtained during life from cases of bacterial endocarditis were shown to be related to green forming streptococci as mutants.^{3,4} When a pure culture of bacteria has been isolated on more than one occasion from an individual, it has invariably proved to consist in organisms responsible for the valvular infection. It is suggestive that the bacillus presented here was likewise the primary agent of the terminal disease.

CONCLUSIONS

A bacillus that fits best into the order Actinomycetales was discovered by culture in the blood of an individual with bacterial endocarditis. A description of this organism was not found elsewhere.

NOTE. Advice and assistance were gratefully accepted from Dr. George F. Dick and Miss Helen Van Sant.

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THE EFFECT OF SALTS OF CITRIC ACID ON SERUM CALCIUM IN THE ALBINO RAT*

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SINCE sodium citrate has been shown to be lethal to the spirochetes of the mouth¹ and has been used successfully in the local treatment of Vincent's infection of the mouth and pharynx, it has seemed of interest to observe the effects on serum calcium of three salts of citric acid.

The salts studied are trisodium citrate, ammonium hydrogen citrate, and ferric ammonium citrate.

Albino rats were used for the calcium studies because of the convenience in handling this species and because white rats were made available to us through the courtesy of the Anatomy Department of Emory University Medical School. The animals were descended from a strain heterogeneous in origin but inbred for several years in the Anatomy Department laboratory. A considerable degree of uniformity has been attained in size and longevity; the mortality rate is low and fertility is high. Animals used in these experiments were from eighteen to thirty months in age and weighed from 200 to 300 gm. Both sexes were used indiscriminately.

Injections were made under ether anesthesia via a tail vein with a graduated tuberculin syringe and a twenty-eight gauge needle, as rapidly as a smooth, steady flow into the vein could be induced. The salts were administered in the form of aqueous solutions usually of 20 per cent strength. The minimum lethal dose was roughly determined for each salt, and in each case was seen to vary over a short range of dosage in different individuals. The cause of this variation was partly dependent on individual physiologic variations among the rats but also may have been affected by technical variations which were not eliminated, since the calcium levels found in each set of experiments were consistent and the data supported the assumption that in all cases the conditions of the experiments were sufficiently uniform for our purposes. Technical variations which may have affected the amount of the minimum lethal dose include the time consumed in making the injection, the possible leakage from the vein into the tissues and the extent of dilution of the blood where different amounts of the solution were given to rats of different weights. The findings with reference to the minimum lethal dose are presented in Table I. It will be seen that this dosage covers a not very wide range of concentrations for all three salts, under the conditions of our experiments.

Blood was drawn from the heart under ether with an ordinary 1 or 5 c.c. syringe and a 2 inch hypodermic needle. Serum was obtained in the usual

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way. The time which elapsed between the completion of the injection and the drawing of blood varied from ten to forty minutes. Within this range the calcium level had no relation to the time interval, low and high values for serum calcium being found at either extreme.

TABLE I
MINIMUM LETHAL INTRAVENOUS DOSE OF CITRATES FOR RATS

SALT	MG SALT/CM RAT	EFFECT ON ANIMAL
Sodium citrate	1.015	Tetany for about $\frac{1}{2}$ hour, death
Ammonium citrate	1.920	Slight tetany with apparent recovery after about 10 min., death after about three hours
Ferric ammonium citrate	1.0	No tetany, death after about one hour

For the determination of serum calcium the micromethod of Hirschfelder and Seles² was used. This method is a modification of the well known Kinner-Tisdall method. The micromodification requires only 0.2 cc of serum and quantities of reagents are correspondingly reduced. Practice is required in observing the end point of the titration with diluted potassium permanganate (0.001N) and the results here obtained are believed to exceed somewhat the true values owing to error in titration. This error, however, is believed to be consistent throughout the determinations, since all were made by one individual and good agreement was obtained between duplicate titrations.

The results of the work on serum calcium are presented in Table II.

TABLE II^a
SERUM CALCIUM IN NORMAL RATS AND IN RATS INJECTED WITH SALTS OF CITRIC ACID

NO. OF ANIMALS	HISTORY	SERUM CALCIUM (RANGE OF VALUES FOUND)	SERUM CALCIUM (AVG.)	COMMENT
3	Normal	11.0-15.9	12.6	
"	" 5 days after injection	11.3-15.5	13.5	2 rats injected with sodium salt, 1 rat injected with ferric salt. No tetany at any time.
7	Sodium citrate 0.8310 mg./gm rat	4.0-11.5	7.2	Marked tetany prolonged beyond 10 min. Death of rats 30 minutes.
5	Ferric ammon. citr. 1 mg./gm rat	11.2-4.8	11.3	No tetany.
6	Ammonium citr. 0.9818 mg./gm rat	13.9-15.1	14.2	Slight tetany with recovery within 10 minutes.

^aSerum calcium expressed as mg. per 100 cc. serum, i.e. as mg. per cent.

The normal values found are somewhat higher than the figures for the normal reported by Shohl and coworkers^{1,4} in two papers, whose normal range was from 10.4 to 13.8 mg. per cent. McCloy and Koels⁵ report an even lower range for normal serum calcium in rats, i.e., 4.7 to 11.1 mg. per cent. If the results cited from both groups of workers are accepted, it is seen that in the present investigation only one instance of a calcium value below the lower limit of the normal range was observed. However, the calcium values found and the effect of the salt on voluntary muscles was correlated here, where comparable quantities of each salt were administered.

From the above data of Table II, it is seen that there is some relation between the onset of tetany and a lowered serum calcium level, although a low calcium level is not always seen where tetany occurs. In these experiments, a drop in the serum calcium below 11 mg. per cent, following the injection of sodium citrate, was accompanied by marked tetany. After ammonium citrate, slight attacks of tetany generally occurred with no fall in serum calcium, but the attacks were of short duration with recovery to normal movement even when death eventually ensued. Ferric ammonium citrate induced no fall in serum calcium and no signs of tetany. Shohl and coworkers⁶ have observed an association of tetany in rats with the reaction of the salt mixture given with synthetic diets, where a neutral diet was always accompanied by tetany and an average serum calcium level of 11.9 mg. per cent. Alkaline diets were only occasionally associated with tetany, although serum calcium fell to values between 7.7 and 8.2 mg. per cent. With acid diets, often associated with rickets, tetany was never observed, although serum calcium had a value of 9.0 to 9.8 mg. per cent. The value of the Ca:P ratio may be involved in the production of some types of tetany and the value of serum calcium alone cannot be relied on as an indication of the imminence or improbability of an attack of tetany, although tetany probably should be expected if serum calcium falls to a sufficiently low level, regardless of blood phosphorus.

SUMMARY

1. Normal serum calcium in white rats was found to range between 11.0 to 15.9 mg. per cent.
2. Three salts of citric acid were studied as to the effect on serum calcium and the onset of tetany after intravenous injection in white rats.
3. Serum calcium was markedly lowered after injection of sodium citrate but showed no deviation from the normal range of concentration after injection of ferric ammonium citrate and of ammonium citrate.
4. Tetany followed injection of sodium citrate exceeding 0.9 mg. per gm. rat; slight tetany, invariably followed by recovery of normal movement, followed intravenous doses of ammonium citrate exceeding 1 mg. per gm. rat; injection of ferric ammonium citrate was never observed to produce signs of tetany.
5. The minimum lethal dose of each salt for the albino rat was roughly determined.

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ONSET AND COURSE OF EPIDEMIC MENINGITIS AS RELATED TO FLUCTUATIONS IN TEMPERATURE AND BAROMETRIC PRESSURE*

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SEASONAL variations in the incidence of diseases transmitted through the medium of the upper respiratory tract with concentration of cases during the colder months has long been appreciated and taken cognizance of. It was deemed advisable to subject this gross accepted fact to more detailed analysis. With this in view a duly study of weather conditions was made and correlated with the incidence of epidemic meningitis in Sangamon County, Illinois during 1935.

In order to logically lead up to the presentation which is to follow, it will be necessary to state some of the salient features relevant to the transmission of the meningococcus and its mode of invasion of the body.

It is well known that persons associated with the patient rarely acquire the disease. These people, however, frequently become carriers and disseminate the infective agent to others. Healthy passive carriers outnumber the cases of epidemic meningitis 10 or 30 to 1. It has been found that the percentage of carriers varies from 1 per cent in individuals who have had no known contact with cases to 80 per cent among intimate contacts under military conditions. The healthy passive carrier therefore is considered to be the main source of infection. The carrier rate is higher in the cold months than in the summer.

The infection itself is divided into three stages: the first, a localization in the upper air passages commonly the nasopharynx, the second, an invasion of the blood stream, the third, metastatic localization commonly in the meninges, but also in the joints, pericardium, endocardium, lungs, skin and other foci. It of course may not be possible to distinguish three such stages in every case.

It has often been a matter of conjecture as to why so many people may harbor the meningococcus in the nasopharynx and so few develop the clinical disease. This question would be directly referable to the invasive powers of the organism, tissue permeability, and resistance of the host. It is not the purpose of this paper to attempt to explain all the factors but merely to present evidence as to one possible influential condition favoring the invasion of body tissue by the meningococcus.

Petersen¹ has previously shown the significance of the passage of atmospheric fronts for the penetration of the meningococcus. Thus with a polar front (or the unusual development of a tropical front), the individual develops a distinct "pressor episode" with general or localized areas of vascular spasm and anoxemia. This period of vascular spasm is followed by either general or local stimulation of the organism. During this latter phase bacteria are more likely to

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pass the mucous membranes, or, if circulating in the blood stream, may become localized in regions previously anoxemic because the vascular endothelium has here become more adhesive. Regions of terminal vascularization—the anoxemia having been more pronounced—will be seemingly selective in their greater disposition to infection under such conditions.

In studying the possible relation of meteorologic alterations to tissue invasion and localization of the meningococcus, the data for analysis were obtained from the epidemiologic cards available in the Division of Communicable Diseases of the Illinois Department of Public Health. The cases of epidemic meningitis studied were those occurring in Sangamon County during the year 1935.

A meteorograph was prepared for the periods in which cases occurred. The solid line in the middle of the graph indicates the mean daily barometric

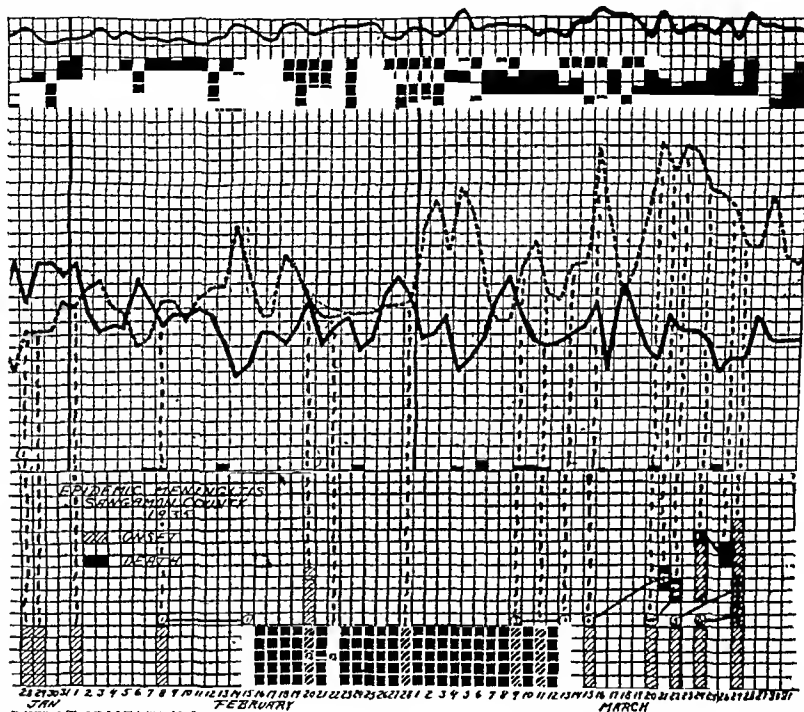


Fig. 1.

pressure, and the dotted line the mean daily temperature. The solid areas at the top signify percentage of cloudiness and the continuous solid line at the extreme top, the daily wind velocity. The date of onset of cases is indicated by the striated blocks, and the date of death, where fatal issue resulted, by solid blocks. Each figure represents one case. The date of onset and death in the fatal cases is signified by corresponding numbers connected with a solid straight line. The graphs and their interpretation are now presented.

The cases of January 28 and 29 both follow immediately in the wake of a polar crest that reached its maximum on January 27.

A case occurs on February 1 during a minor polar infall but the general tendency is for rising temperatures.

The first fatal case has its onset on February 9 again immediately in the wake of a polar crest which occurred on February 6 and 7. The patient dies on February 15 at the beginning of a very sharp polar front.

Two cases have their onset on February 20 during the next succeeding front and one of these dies on February 22 at the polar infall initiated on February 20.

One case occurs on February 28 at a tropical front but actually on the day following a great barometric crest.

A case (5) occurs on February 9 at a period that is quite similar to the preceding and this patient dies one day after the succeeding polar episode of March 12.

One case during the stormy period of March 11. This is coincident with a polar infall.

A case (1) occurs with a barometric crest on March 7 and dies with the polar front of March 21.

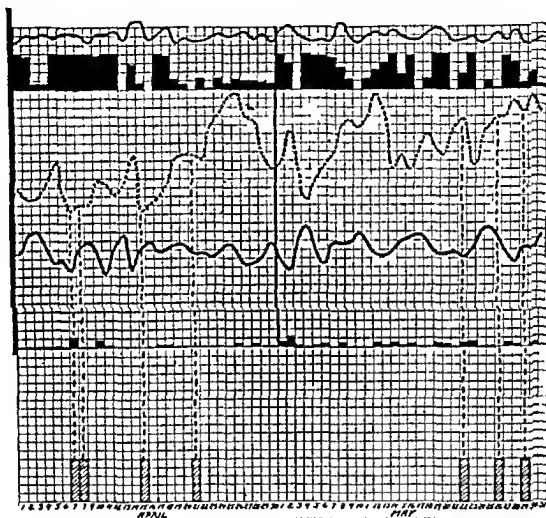


Fig. 2

A case (4A) occurs at the tropical period of March 20 and dies immediately in the wake of a barometric crest that was associated with death in the preceding case.

A case (5) originates at the same time and the patient dies during the polar episode that began on March 23 and extended to March 28.

Two cases (6 and 7) begin at the polar front on March 24. Patient (Case 7) dies on March 26 and another patient (Case 6) dies on March 27 as there is a renewal of the polar effect.

Another case is reported with the polar front of March 27.

Two cases on April 7 and 8, respectively, at the time of a stormy period associated with falling temperatures, falling barometer and heavy precipitation (partially occluded cyclone).

Another case occurs with the polar infall of April 15.

A case occurs during a polar episode evident during the time when there was rising temperatures.

A case occurs with the tropical crest of May 22.

A case occurs immediately in the wake of the barometric crest of May 25.

A final case occurs that is coincident with the polar infall of May 29.

Case 8 begins with the tropical crest of November 9, but at a time when the barometric curve indicated a coincident polar effect. The patient dies following the polar infall of November 14.

A case occurs at a tropical period of November 20. This is one day after an extension of the barometric high which reached its crest on the sixteenth of November.

A case occurs at a polar front on November 22.

Two cases occur with the tropical fronts of November 25 and 27, the first immediately after the great barometric crest of November 24, and the second with the distinct extension of the barometric crest evident in the graph.

A case occurs immediately after the barometric crest of the fourth of December.

A case occurs at the polar crest of December 11.



Fig. 3.

Case 9 begins immediately in the wake of this same crest and the patient dies with the final cold wave of the month.

It is probable that bacterial penetration of tissue is of common occurrence. Unless the organism concerned is of high virulency or the local resistance is low, there probably results nothing more than reticulo-endothelial fixation of the invader with subsequent destruction. It may be assumed that in some instances the organism so fixed is not destroyed, but remains quiescent. If such be the case, the occurrence of a sudden pressor episode, meteorologic or otherwise, may cause the mobilization² of the fixed bacteria and their being thrown into the circulation.

The effect of the pressor episode correlates rather nicely with the three stages previously described in the development of epidemic meningitis. First we have

the deposition of the bacterium in the nasopharynx as a result of contact with case or carrier. Penetration of tissue must be assumed to exist markedly enhanced after the pressor episode. The degree of such an effect will vary with the individual. In some cases there will be fixation and destruction of the bacterium. In others there will be temporary quiescence. With an additional pressor effect, mobilization can occur, associated with dissemination through the medium of the circulation. This is in accord with our conception of a stage of bacteremia.

Since a reflex vasoconstriction occurs in the brain following closely the similar phenomenon observed associated with the chilling of skin and mucous membrane, with associated anoxemia and increased adhesiveness of the vascular endothelium, subsequent deposits of the meningococcus in the meninges, from the circulation would be favored.

SUMMARY

The study of the onset of meningitis in Sangamon County, Illinois during the year 1935 has confirmed the evidence previously presented by Petersen concerning the significance of the passage of atmospheric fronts for the invasion of the body by the meningococcus.

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LABORATORY METHODS

HOT AIR STERILIZATION AND ITS PRACTICAL APPLICATION IN BACTERIOLOGIC LABORATORIES*

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THE difficulties in obtaining good results in bacteriologic work in laboratories have taught us to lay particular stress on thorough sterilization of the glassware intended for experimental purposes. Various methods for sterilization have been employed, yet not one of them has proved to be completely satisfactory.

To render the glassware sterile means to kill all bacteria present in the glassware. Bacteria can be killed by diverse agents. All agents must act upon bacteria by chemical or physical processes in such a way as to render them dead. The bacterial cell is considered dead when there is a permanent cessation of all the anabolic and catabolic changes which under normal conditions manifest themselves in it, followed by a decomposition of the resultant fixed substances. It therefore constitutes such a state where neither the growth, nor the multiplication of the bacteria is any more possible.

The most valuable agent for the destruction of bacteria is heat. There is a marked difference between dry and moist heat. This difference is of great practical value, since wet air (water) has a higher potency for killing bacteria resistant to heat in a shorter period of time. This is very important for disinfecting purposes.

The bactericidal action of dry heat takes place at very high temperatures. Bigelow and Esty have demonstrated that the destruction of spores by heat is not instantaneous but a gradual process during which the spores are progressively injured and finally destroyed. An organism at the temperature above the maximum for its growth cannot survive, and the higher the temperature to which it is exposed, the sooner will it be killed.

Lister in 1878 devised the hot air sterilizer in order to destroy all organisms present in dust, air, water used for washing, and in the packing material of glassware. Since among the organisms found in the air, dust, packings, etc., there are spore formers, it is necessary for the proper sterilization to destroy not only the nonspore-bearing bacteria but also the spores which have, so to say, a protective function. To accomplish their death it becomes essential to study the nature of the spores, their environmental conditions, normal habitat, and the optimal temperature and time for their growth and destruction.

Reichenbach in 1908 demonstrated that various spores show a variable degree of resistance to heat and that the survival of certain spores at tempera-

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tures at which others are destroyed can be traced to individual resistance. The law of mass action cannot be applied to spores, since the destruction of spores in different species takes place according to different laws.

Besides the variation in resistance of spores of different species, the resistance may also differ in the various developmental stages of the spores of the same species, as well as with various environmental conditions. Old spores are less resistant to heat than fully developed young ones. Bigelow found that young spores about one month old have the highest thermal resistance. On the other hand, the vegetative forms are less resistant to heat than the fully developed spores. Again, dry spores manifest a slight increase in heat resistance as a result of dehydration, while the same spores in a moist condition stored exactly the same length of time would exhibit essentially the resistance of young spores. Pasteur has demonstrated that spores remain alive in a dry form for twelve or more years.

Various theories have been advanced to explain this high resistance of spores to heat. At present it seems reasonable to assume that it is due to: (1) the spore membrane which acts as a protective structure; (2) the low water and salt content of its protoplasm as compared with that of the vegetative cell.

The abundance of spores is not always an indication of the presence of highly resistant forms. Nevertheless, a larger amount of spores will require a higher temperature and a longer period of exposure for them to be killed. It has long been recognized that a very definite relationship exists between the time necessary to destroy any given suspension of organisms and the temperature to which the suspension must be exposed. Williams, therefore, introduced the term "thermal death time" in preference to the older term "thermal death point."

The behavior of spores when subjected to heat has been and is continued to be studied by numerous investigators. Tyndall in 1877 demonstrated that the spores of certain bacteria have two phases, one being thermolabile and destroyed at 100° C. in five minutes, and the other, what he calls "the germ of the bacterium" being thermoresistant to an almost incredible extent. Ferdinand Cohn in the same year arrived at the same conclusions by different methods. In order to kill the former and overcome the latter, Tyndall developed a method of fractional sterilization by discontinuous heating known as tyndallization.

Another interesting phenomenon has been observed in the so-called "skip-stop" stages. These skip-stops appear during or after three months of incubation and are believed to be the result of an uneven distribution of the spores. This phenomenon assumes special significance in sterilization and can be eliminated by a sufficiently prolonged incubation and by a careful preparation of a uniform suspension with an even distribution of the organisms.

The author of this paper has undertaken an extensive study of hot air (dry) sterilization, dealing with the processes taking place in the hot air sterilizer, and also of the heat resistance of *Bacillus subtilis*, since it is frequently found in the glassware and is believed to be the strongest or most stubborn organism to heat destruction. This paper is divided into two parts: the first part dealing with the functions and sterilization properties of the sterilizer,

and the second part with the behavior of *Bacillus subtilis*, when submitted to dry heat. For this purpose a series of tests over a period of ten months was performed and the results recorded in the charts and tables.

The hot air sterilizer used for these tests is constructed as follows: It is made of supermetal (galvanealed iron) and is provided with three metal walls. Between the outer and the second wall which form one side of the flue, there is a layer of one inch asbestos insulation; then an inner metal wall forming the chamber and the other side of flue. Beneath the oven are mounted three gas burners. The compartment is perforated on top and bottom. Flues are arranged at sides, back, and top, and dampers are provided. Gas burners are baffled, some of the products of combustion passing around the sterilizing chamber, and others passing through it, providing a forced air circulation necessary for an even temperature. The oven is fitted with an automatic thermostatic control with an expansible element extending half way across the chamber at the top. The individual burners are equipped with gas cocks and air mixers, but all are controlled by the thermostat. The top of the sterilizer is fitted with three tubulatures where thermometers are inserted. The forced air circulation distributes the temperature. In order to find out whether the temperature is evenly distributed to render all glassware placed in the sterilizer sterile, minimum and maximum thermometers were placed inside to depict the various temperatures at various angles of the sterilizer. The sterilizer thus constructed was set into operation and an extensive study of the time-temperature relation was made. Observations were first made with an unloaded sterilizer, the initial temperature being approximately 20° C. (Chart 1). Readings were taken at five-minute intervals. At the end of two hours the gas burners were turned off and the thermometers registered the following temperatures: the right one 165°, the left one 180°, and the center one 170° C.; the minimum and maximum thermometers, 175° C. Therefore with the sterilizer empty, the inside temperature rapidly rises, while in the presence of a load, as soon as it reaches 150°, the thermostat comes into play by cutting down the flame of the burners.

The experiment was then repeated with a moderate load (Chart 2) consisting of 4 square crates of Petri dishes, 6 crates of test tubes, 2 crates of bottles and packages of pipettes wrapped in paper. The burners were lit at 9:45 A.M. with the initial temperature of about 20°, and at the end of two hours the gas was turned off. The right thermometer registered 172°, the left 175° and the center 160° with the inside temperature of 173° C.

Thus with this moderate load it took forty minutes for the load temperature to reach 153°, the temperature at which suitable sterilization begins. It is also important to note that with this load both the inside and outside temperatures closely approximate each other throughout the length of the experiment. Therefore, the outer thermometers could be used as a fair index of the inside temperature, eliminating the necessity of frequent opening of the sterilizer while it is in process of sterilization.

The third test was performed on an overloaded sterilizer (Chart 3). The load consisted of ten large crates of Petri dishes; 2 crates of test tubes; of many paper wrapped articles. The upper shelf of the sterilizer was over-

loaded with packages of pipettes, wrapped in paper, and piled up in bundles. The sterilizer was started at 10 25 A M. At 11 10 A M, i.e., at the end of forty-five minutes of operation when the outside temperature reached 155° at the right thermometer 163° at the left one and 150° at the center thermometer,

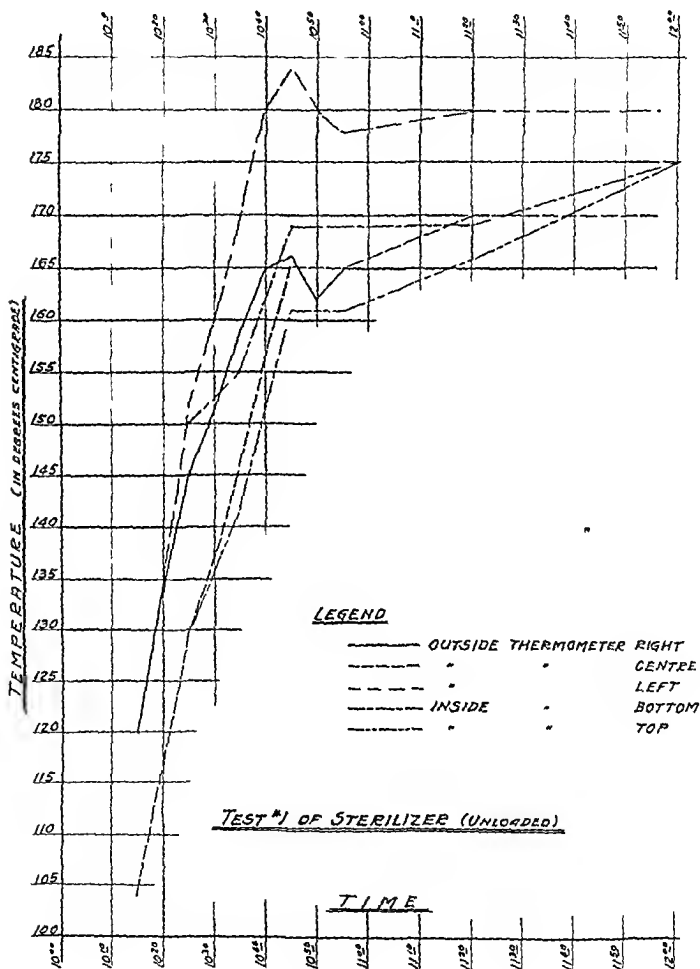


Chart 1

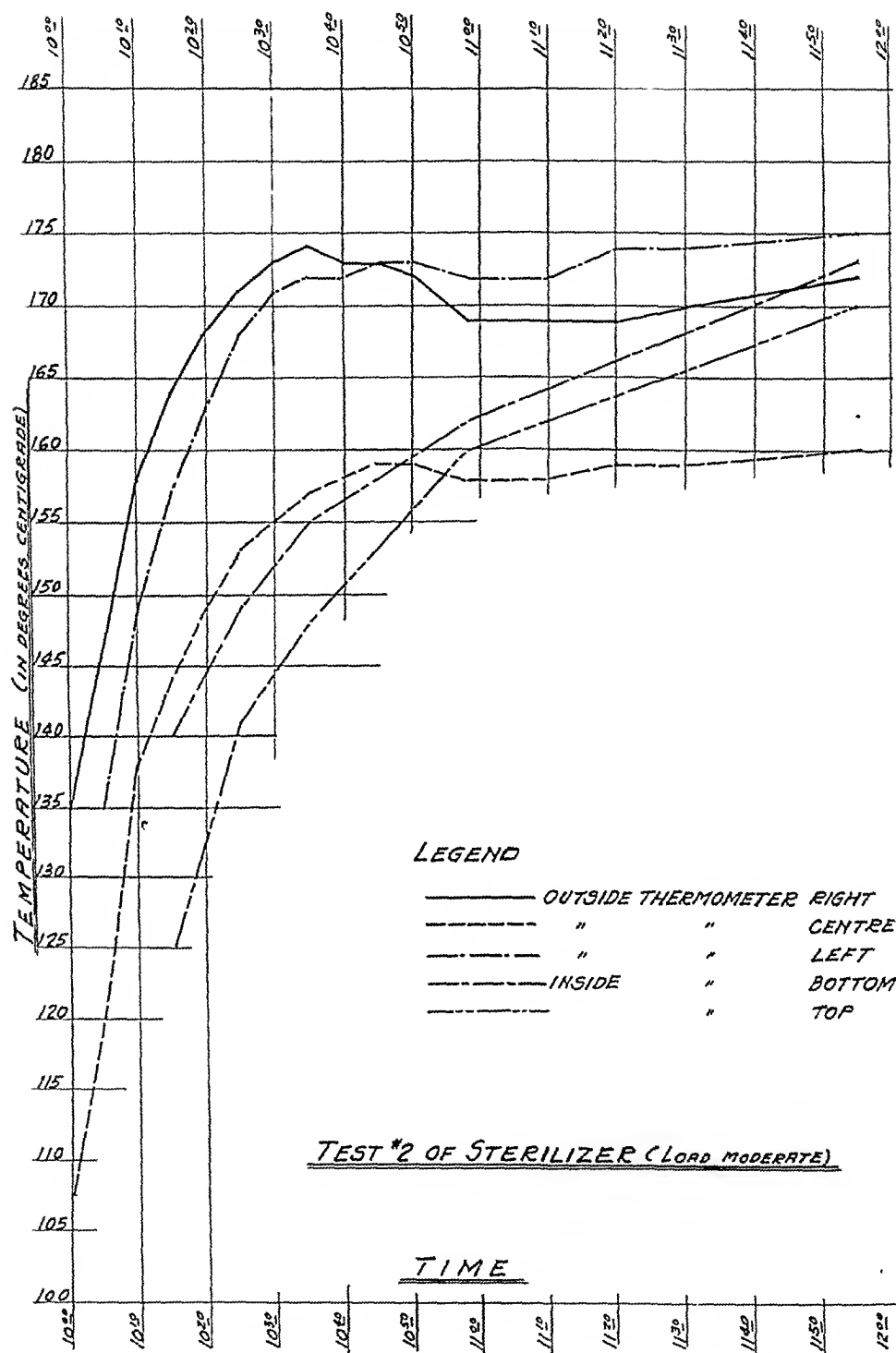


Chart 2.

the inside thermometers registered 125° between the Petri dishes and 105° in the bundles of the pipettes (paper). At the end of two and one fourth hours the gas burners were turned off and the following temperatures found at the

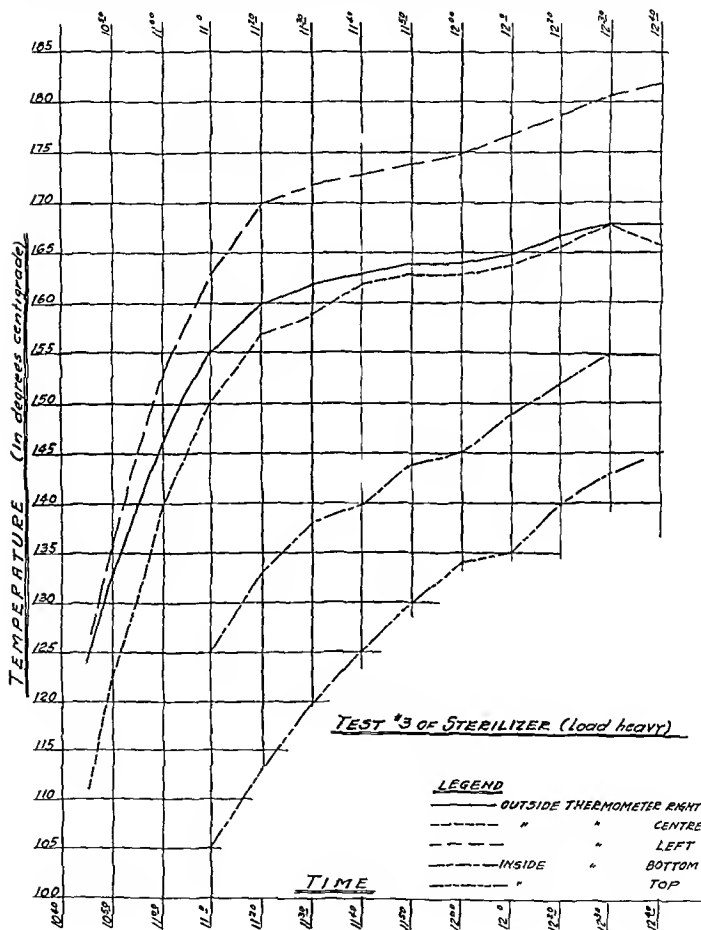


Chart 3

right 168° , left 182° , and the center 166° . The inside temperatures were 155° between the crates, 145° within the paper wrappings of the pipettes and 150° between the Petri dishes

It is evident from these observations that with an overload the time of sterilization must be considerably prolonged, and even when the center thermometer registers 150° C. or thereabout, the inside temperature still remains

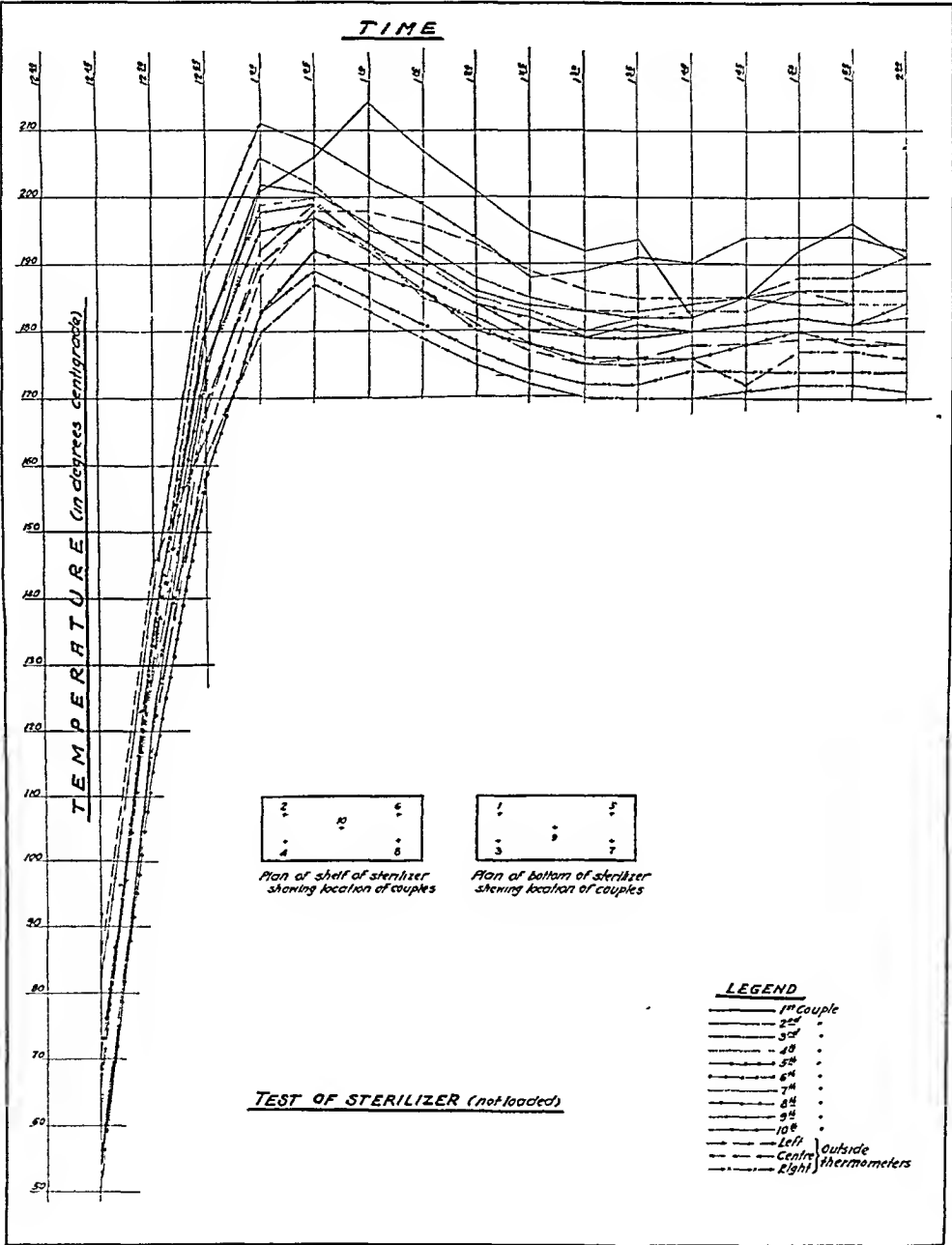


Chart 4.

for a long time below this point. Besides, there is also a difference in the temperature in various points of the sterilizer as well as within the various containers of the articles to be sterilized. As a control, a series of similar tests

The findings of these experiments were as follows: if the sterilizer was unloaded, it maintained an approximately even temperature, but when the sterilizer was loaded, there occurred two temperatures, one being the air temperature and the other the load temperature.

The above-mentioned experiments have shown that in order to assure proper results the following principles must be adhered to in operating this sterilizer.

TABLE I*

140° C. ONE HOUR VARIOUS ORGANISMS THERMAL DEATH TIME

TYPE OF GLASSWARE USED IN TEST	MATERIAL SEEDED	POSITION OF GLASSWARE IN RELATION TO LOAD	CHARACTER OF LOAD	POSITION OF LOAD IN STERILIZER	FINDINGS
8 ounce bottle	Subtilis X	In crate	Crate of T. B. tubes	Back top, right	Free spores
Test tube	Dust	Attached to crate	Crate of centrifuge tubes	Front bottom, center	Long chains
Petri dish	Dirt A	On top of crate	Crate of bottles	Front, top shelf	Free spores
4 ounce bottle	Subtilis S	Between crate and wall of sterilizer	Crate of Petries	Center, bottom	Free spores
Petri dish	Staphylococcus	On bottom of crate of tubes	Crate of potato tubes	Front, bottom	No growth
125 c.c. flask	Dirt B	In crate	Crate of small bottles	Center, top	Short bacilli
Petri dish	Subtilis X	Center of crate	Crate of Petries	Front, bottom	Free spores
Potato tube	Subtilis S	In crate	Crate of inverted tubes	Front top, left	Spores
Test tube	Dirt A	In rack	Rack of Smith fermentation tubes	Center, bottom	Cells in pairs
Petri dish	Staphylococcus	Between pipettes	Packages of pipettes	Center, top	No growth

*NOTES: Subtilis X, pure culture isolated from hay.
 Subtilis S, stock culture used in our laboratory.
 Dirt A, from our animal house.
 Dirt B, from the street.
 Dust, collected in the laboratory.
 Staphylococcus, stock culture.

These various types of glassware with planted organisms from above-mentioned material were sterilized at 140° C. for one hour. Extract broth and extract agar were added and incubated at 37° C. for twenty-four and forty-eight hours. All the bacteria survived, except staphylococcus cultures which were killed.

A. *Regarding the Loading of the Sterilizer.*—The sterilizer must not be overloaded. Only a moderate load of approximately 10 crates or its equivalent may be placed in it, so as to permit a free passage of heated air around, under and through the articles. Paper wrappings or packings are to be avoided as much as possible, since paper forms a dead wall and interferes with the penetration of the hot air into the load.

B. *Regarding the Spacing of the Material.*—The load in the sterilizer should be arranged so that the articles should not rest on each other, nor touch the sides or back of the sterilizer. Pipettes are best placed on the upper shelves of the sterilizer in order to avoid burning of the paper wrappings. They should be scattered loosely and not piled up in bundles, so as to assure free passage of air between the packages. On the other hand, pipettes in metal containers may be placed on the bottom, or any part of the sterilizer, since metal being

a good conductor of heat will not disturb the process of sterilization. Special care should be taken in sterilizing Petri dishes. It is bad practice to place the Petri dishes in a crate lined with paper. Instead they are to be placed in crates and only the top of the crates covered with paper.

C Regarding Operation of the Sterilizer—When the sterilizer is started and the gas burners are properly lit, the thermometers will begin to register the inside temperatures, the center one carrying the load temperature, and

TABLE II*

150° C FIFTEEN MINUTES

BACILLUS SUBTILIS G (HAY)

THERMAL DEATH TIME

TUBE	POSITION OF TUBE IN RELATION TO LOAD	CHARACTER OF LOAD IN STERILIZER	POSITION OF LOAD IN STERILIZER	FINDINGS	
				MACROSCOPIC	MICROSCOPIC
1	In crate	Crate of bottles	Center, bottom	Granular pp	Long chain formation
2	Under paper cover in crate	Crate of Petri dishes	Center, front	Heavy pellicle	Long chains
3	On top of crate	Crate of potato tubes	Center, bottom	Cloudy	Long bacilli
4	Tied to crate	Crate of Petri dishes	Front, upper right	Cloudy	Short bacilli
5	On top of crate Tube wrapped in newspaper	Crate of bottles	Center, bottom	Slightly cloudy	Bacilli and threads
6	Attached to crate, 4 inches from bottom	Crate potato tubes	Center, bottom	Cloudy	Cells in pairs rounded edges
7	Attached to crate, near wall of sterilizer	Crate bottles	Front left, bottom	Granular pp	Short bacilli
8	Lying on bottom of crate wrapped in paper	Square crate tubes	Front, right	Slightly cloudy	Long chains
9	Top of crate	Crate of Petri dishes	Rear left, top	Cloudy	Short bacilli
10	On crate of bottles	Crate of bottles	Rear left, bottom	Pellicle	Long chains
11	Between wall of sterilizer	Crate of Petri dishes	Upper left, top	Cloudy	Cells in pairs
12	Between two crates	Crate of tubes	Rear right, bottom	Granular pp	Short bacilli

*NOTES The tubes with the tested material (*Bacillus subtilis* isolated from hay) were kept in the sterilizer for fifteen minutes at 150° C. They were then removed from the sterilizer and with extract broth added to them incubated at 37° C for twenty four hours.

the two end ones representing the air temperature of the oven. With the sterilizer in full operation, readings from the three thermometers are taken simultaneously to determine the length of time necessary for the load temperature, as registered by the center thermometer, to rise to 150° C. Naturally, this will vary depending upon the extent and character of the load. When this temperature is reached, actual sterilization is considered to begin and is continued for one and one half hours. At the end of this period the burners are turned off, the doors being gradually opened to permit the load to cool.

After having established thus the requirements for successful operation of the sterilizer, a series of tests were performed to determine the degree of resistance of bacteria to dry heat. As it was indicated previously, the investi-

gation was centered mainly on the *Bacilli subtilis*, since these are the most frequently met with contaminating agents in the laboratories, being at the same time highly resistant to heat. Observations were also made with staphylococcus taken from stock cultures used in our laboratory. The sources from which *B. subtilis* was isolated were numerous, so as to include as much as possible the usual habitats of the organisms. They consisted of hay, dust, street dirt, and dirt from the animal house of our laboratory, as well as spores

TABLE III^a

150° C. THIRTY MINUTES BACILLUS SUBTILIS (HAY) THERMAL DEATH TIME

TUBE	POSITION OF TUBE IN RELATION TO LOAD	CHARACTER OF LOAD IN STERILIZER	POSITION OF LOAD IN STERILIZER	FINDINGS	
				MACROSCOPIC	MICROSCOPIC
1	Tied to crate of Petri dishes	Crate of Petri dishes	Front center, upper left	Pellicle	Long bacilli
2	On top of crate	Crate of tubes	Back bottom, left	Pellicle	Cells in pairs
3	Top of crate. Tube covered with paper	Crate of tubes	Back bottom, right	Cloudy	Long chains
4	In crate. Tube covered with paper	Crate of 4-ounce bottles	Front, center	Granular pp.	Free spores
5	On top of crate	Crate of bottles	Back center	Clear	Negative slide
6	Between crate and wall of sterilizer	Crate of Petri dishes	Front, left	Clear	Negative slide
7	On top of crate. Top of wrapped in paper	Crate of matched plates	Front, upper right	Heavy pellicle	Free spores
8	Under cover of crate	Crate of matched plates	Front, upper right	Pellicle	Short bacilli
9	Top of two crates	Crates of centrifuge tubes	Top, center	Cloudy	Free spores
10	On crate	Crate of tubes	Front, lower left	Pellicle	Short bacilli
11	In crate	Crate of tubes	Front, lower right	Pellicle	Chain formations
12	Between two crates	Crate of tubes	Front, lower right	Pellicle	Chain formations

*NOTES: The tubes were smeared with *Bacillus subtilis* and kept in sterilizer for thirty minutes at 150° C. Extract broth was added and incubated for forty-eight hours at 37° C. After ninety-six hours of incubation one of the clear tubes that showed no growth became cloudy and produced heavy pellicle.

from stock cultures. Besides, efforts were made to approximate in the laboratory as closely as possible the normal environmental conditions of the organisms to produce abundant growths.

Hay infusion was prepared and tubed. The tubes were divided into four sets. Nine tubes of each set were boiled for fifteen minutes, thirty, forty-five minutes, and one hour, respectively. The tubes were subjected to the preliminary boiling for various lengths of time to eliminate the vegetative forms and less resistant bacteria. Following it the tubes were incubated in groups of three of each set at 25°, 30°, and 37° C., respectively, to determine the optimum temperature for their growth and development. When an adequate growth appeared it was transferred to beef extract agar slants and reincubated, thus

stimulating the growth, smears having been taken before and after the addition of the medium to identify the organisms and the abundance of their spores

Another source for obtaining highly resistant spores was dust. Sterile test tubes with the cotton plugs removed remained uncovered in the laboratory rooms for twenty four hours to collect as much dust as possible. Swab sticks were rubbed with dust and put into each tube.

TABLE IX *

150° C FORTY FIVE MINUTES *BACILLUS SUBTILIS* (HAY) THERMAL DEATH TIME

TUBE	POSITION OF TUBE IN RELATION TO LOAD	CHARACTER OF LOAD IN STERILIZER	POSITION OF LOAD IN STERILIZER	FINDINGS	
				MACROSCOPIC	MICROSCOPIC
1	Between bottles Bot tom of tube covered with brown paper	Square crate of bottles	Front, top, cen ter	Pellicle	Free spores
2	Between bottles Top of tube covered with brown paper	Crate of bottles	Front, upper right	Pellicle	Free spores
3	Between two crates Entirely wrapped with brown paper	Crates of Petri dishes	Upper front, top shelf	Pellicle	Free spores
4	Between T B tubes	Crate of T B tubes	Back bottom, center	Clear	Few bacilli
5	Between 2 ounce bot tles	Crate of bottles	Front, upper left	Clear	Few bacilli
6	Top of tubes	Crate of potato tubes	Front bottom, center	Clear	No growth
7	Top of 4 ounce bot tles	Crate of bottles	Back bottom, left	Pellicle	Spores
8	Attached to crato	Crate of large bottles	Back bottom, left	Clear	No growth
9	Between crate and wall of sterilizer	Crate of potato tubes	Front, upper shelf	Cloudy	Long bacilli
10	Between two crates	Crates of tubes	Back, bottom	Clear	Few bacilli
11	Top of Petri dishes	Crate of Petries	Front center	Pellicle	Long chains
12	In crate Wrapped in newspaper	Crate of miscel laneous glass ware	Front bottom, right	Cloudy	Chain forma tion

*NOTES The tubes with *Bacillus subtilis* isolated from hay were sterilized for forty five minutes at 150° C. Extract broth was added to the tubes. After prolonged incubation tubes with few bacilli produced heavy growths. Tubes with no growth did not show any changes.

To obtain suitable spores in addition, dirt was also taken from the street and incubated. The dirt from the animal house containing animal (rabbit and guinea pig) hairs and feces, was put into 10 cc of beef extract broth and dilutions made of 1/10, 1/20, 1/40, 1/80, 1/100. These dilutions were incubated at 37° C for one hour and transferred to beef extract agar pour plates and incubated for twenty four and forty eight hours at 37° C. It was noticed, however, that the growth was most abundant in the dilutions of 1/10 and 1/20. In the higher dilutions the number of colonies was considerably decreased. This source was used for the isolation of different types of organisms found in the soil.

The pure cultures of *B. subtilis* isolated from hay were smeared on walls of sterile test tubes under aseptic conditions and these together with organisms

obtained from the other sources as well as staphylococcus cultures were subjected to the same environmental conditions as the glassware placing them in the sterilizer in various positions indicated by the tables and sterilized applying varying temperatures and periods of time

Besides test tubes, Petri dishes, bottles, and flasks of the sizes and descriptions used in laboratories, wrapped in paper and unwrapped were employed for smearing the bacteria to be tested

TABLE V*

150° C. ONE HOUR *BACILLUS SUBTILIS* (HAY) THERMAL DEATH TIME

TUBE	POSITION OF TUBE IN RELATION TO LOAD	CHARACTER OF LOAD IN STERILIZER	POSITION OF LOAD IN STERILIZER	FINDINGS	
				MACROSCOPIC	MICROSCOPIC
1	Under cover of crate	Crates of matched plates	Back, upper right	Clear	No growth
2	Top of crate	Crate of Petries	Back top, cen- ter	Clear	No growth
3	Tied to crate. Tube wrapped with newspaper	Crate of matched plates	Back, upper left	Slightly cloudy	Short bacilli
4	Between two crates	Crates of centri- fuge tubes	Back bottom, center	Granular pp.	Free spores
5	Tied to side of crate	Crate of Petries	Front top, cen- ter	Clear	No growth
6	Bottom of crate. Tube completely covered with brown paper	Crate of test tubes	Back bottom, left	Cloudy	Long bacilli
7	Center of crate	Crate of potato tubes	Back bottom, right	Clear	No growth
8	Tied to crate. Top of tube covered with brown pa- per	Crate of Petries	Back bottom, center	Slightly cloudy	Few bacilli
9	In a rack	Rack for Smith fermentation tubes	Front bottom, right	Clear	No growth
10	Tied to metal con- tainer	Metal containers of pipettes	Front bottom, left	Clear	No growth
11	Center of crate. Top of tube cov- ered with brown paper	Crate of flasks	Front bottom, center	Pellicle	Spores
12	Between crate and wall of sterilizer	Crate of bottles	Bottom, right	Clear	No growth

*NOTES Tubes smeared with *Bacillus subtilis* isolated from hay, were kept in sterilizer at 150° C. for one hour. Extract Broth added and incubated at 37° C. for various periods. No changes occurred after one month of incubation.

Bacillus subtilis isolated from hay was carried to twenty generations in order to gradually attenuate their resistance. The material to be tested was exposed to 140° C. for one and one-half hours; to 150° C. for fifteen, thirty, forty-five minutes, one hour, and one and a half hours; 160° C. for fifteen minutes and one hour. After each sterilization the test tubes, Petri dishes, bottles, and flasks with the planted organisms were removed from the sterilizer, beef extract broth and beef extract agar were added to them and cul-

tures incubated at twenty-four, forty-eight, seventy-two, ninety-six, etc. hours at 37° C. and tested for the presence of actively living or dormant organisms.

The organisms found in the samples of dirt from the animal house and the *B. Subtilis* from hay were most resistant to heat. However, all were destroyed at 150° C. for one and a half hours and at 160° C. for one hour.

At 150° for fifteen, thirty, and forty-five minutes most of the spores survived, some though at the longer periods were dormant. Those which were exposed at 140° C. were not killed after one and one-half hours of sterilization.

TABLE VI*

150° C. ONE AND ONE HALF HOURS		VARIOUS ORGANISMS		THERMAL DEATH TIME	
TYPE OF GLASSWARE USED IN TEST	MATERIAL SEED	POSITION OF GLASS WARE IN RELATION TO LOAD	CHARACTER OF LOAD	POSITION OF LOAD IN STERILIZER	FINDINGS
Petri dish	<i>Subtilis</i> X	Top of crate	Crate of potato tubes	Front bottom, center	No growth
Petri dish	Dirt A	Bottom of crate	Crate of Petries	Front top, right	No growth
Petri dish	<i>Subtilis</i> S	Between crate and wall of sterilizer	Crate of matched plates	Front top, left	No growth
Petri dish	Dirt B	Center of crate	Crate of tubes	Back bottom, left	No growth
8 ounce bottle	<i>Staphylococcus</i>	Attached to crate. Bottle entirely wrapped with paper	Crate of Petries	Center, top shelf	No growth
Test tube	Dust	Bottom of crate	Crate of bottles	Back top, right	No growth
4 ounce bottle	Dirt A	In crate	Crate of tubes	Front, top shelf	No growth
Potato tube	<i>Subtilis</i> S	Bottom of crate	Crate of matched plates	Front, bottom	No growth
Potato tube	Dirt B	Attached to crate. Tube wrapped with brown paper	Crate of bottles	Center, bottom	No growth
Petri dish	<i>Subtilis</i> X	Between pipettes	Packages of pipettes	Front top, left	No growth
Test tube	Dust	In crate	Crate of matched plates	Back bottom, left	No growth
300 c.c. flask	<i>Staphylococcus</i>	In crate	Crate of miscellaneous glassware	Front top shelf, right	No growth

*NOTES. *Subtilis* X, pure culture isolated from hay; *Subtilis* S, stock culture used in our laboratory; Dirt A, from our animal house; Dirt B, from the street; Dust, collected in the laboratory; *Staphylococcus*, stock culture.

These various tubes, bottles, flasks, and Petri dishes with the material to be tested were sterilized at 150° C. for one and one-half hours, extract broth and extract agar added and cultures incubated at 37° C. for ninety-six hours and longer. No growth appeared.

SUMMARY

In order to insure absolute sterility, regardless of whether the organisms are more or less heat resistant, the sterilization periods should not be less than one and one-half hours and the temperature should be between 155° to 160° C.

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A COMPARATIVE STUDY OF DEHYDRATION*

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THE acetone method is known to be a short and reliable way of dehydrating tissue. Tertiary butyl alcohol has been used by Johansen (1935) with considerable success as a substitute for ethyl alcohol. He believes that a great deal of the shrinkage which has been blamed on fixation was really caused by ethyl alcohol and the commonly used clearing agents. He is of the opinion "that no blame can be placed upon the fixing fluid if disaster results during postfixation stages. In other words, 'successful' dehydration and infiltration is not dependent upon successful fixation; the two processes are mutually exclusive."

Dioxane (diethylene oxide) has been used with varying results by several investigators among the first of whom were Grunpner and Weissberger (1931) who describe the use of dioxane in dehydrating tissue before sectioning. They transferred the tissue from the fixative through dioxane containing calcium chloride to a mixture of paraffin and dioxane into pure paraffin. Very good results were obtained. Johansen (personal communication) obtained very inconsistent results with the use of dioxane.

Cellosolve (ethylene glycol mono ethyl ether), a commercial product used in the cellulose lacquer industry, was recently introduced as a dehydrating agent, by Frost (1935). Frost was able to dehydrate, stain and mount plant tissue in five minutes.

Other reagents have been suggested as substitutes for ethyl alcohol. Some of these have been used with some success in botanical work but none have come to be widely used in the preparation of animal tissues. In most of the techniques the substitute reagents have not entirely replaced ethyl alcohol and some have even been mixed with ethyl alcohol. Among these substitutes for ethyl alcohol may be mentioned a mixture of ethyl alcohol and *n*-butyl alcohol by Laubaud (1921) and Zinke (1930), *n*-propyl alcohol by Sheridan (1929) and iso-propyl alcohol by Bradbury (1931). Baird (1936) made a comparative study of dioxane, iso-butyl alcohol and ethyl alcohol with chloroform on several animal tissues. These were used in dehydration before embedding and were not employed in the staining process. She obtained the best results with dioxane. Mossman (1934) as well as Buehler and Blakely (1936) discuss the use of dioxane in place of ethyl alcohol and the common clearing agents in the paraffin technique. The latter investigators report that preparations were completed in 48 hours. The method described in the work here reported requires a great deal less time.

METHODS

A comparative study of the dehydrating qualities of 1, 4 Dioxane, cellosolve, tertiary butyl alcohol and acetone have been made on some normal and abnormal human tissues following several fixatives. In all cases control slides were made

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of the same tissue using the familiar ethyl alcohol technique followed by a common clearing agent. The human tissues used were pancreas, kidney, liver, cardiac muscle, cervix uteri, cancer of the breast, cancer of the uterus, and tumor of the neck. Some tissues were removed at autopsy and others at biopsy. It should be pointed out that in the technique described here more emphasis has been placed on securing histologic preparations sufficient for diagnosis rather than for cytologic details. Work is in progress on the latter aspect of the problem.

Fixation—Among the various fixatives used were Bouin's, Orth's, 10 per cent formalin, 20 per cent formalin and hot 100 per cent formalin. Bouin's fluid was perhaps the least successful due to the fact that the tissue must be washed in diluted ethyl alcohol or one of the dehydrating agents for a con-

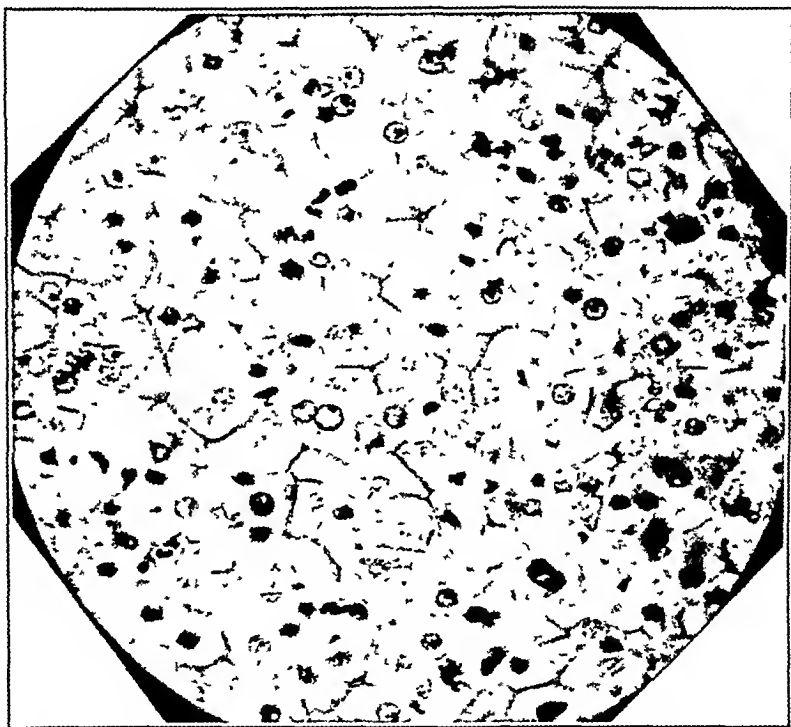


Fig 1—Human liver. Fixed in hot 100 per cent formalin and dehydrated in acetone. Iron hematoxylin

siderable time, to remove the pierce acid, for good staining results. By far the most successful fixative used, from the standpoint of speed, was full strength commercial formalin heated almost to the boiling point, after which the tissue was washed in running water for a few minutes to remove the excess formalin.

Dehydration.—Tissue was transferred directly from the fixative or water, as the case may be, to the undiluted dehydrating agent. It is very important that the dehydrating fluid be perfectly "dry." In the case of acetone and tertiary butyl alcohol chemically pure products were used and they no doubt contained a minimum of water; however, the addition of a small piece of tissue adds enough water to spoil them for further use. For keeping the reagents "dry" generous amounts of anhydrous copper sulphate and calcium chloride

were tried. It was found that copper sulphate was best in all the fluids as it would not only take up more water than calcium chloride but one could tell by the color change when the fluid was too "wet" to use. Calcium chloride was fairly successful with dioxane and acetone but not with tertiary butyl alcohol and cellosolve. The tissue may be suspended in the fluid in cloth bags or placed directly on the layer of copper sulphate.

For complete dehydration from one to one and one half hours is required, depending on the size and texture of the tissue. Two changes were usually sufficient, that is, the tissue was placed in the first container for one half hour and in the second for about an hour.

Although all of the reagents are more expensive per unit volume than ethyl alcohol they can be used repeatedly as long as they are kept "dry." When this is considered it is found that dioxane and acetone, for example, which can be used longer than tertiary butyl alcohol and cellosolve are as cheap as or cheaper than ethyl alcohol.



Fig. 9.—Human liver showing structures in relief. Fixed in hot 100 per cent formalin and dehydrated in acetone. Iron hematoxylin.

Embedding—Tissue was transferred directly from the dehydrating agent to pure melted paraffin with no intermediate steps. In the case of tissue transferred from acetone to melted paraffin, bubbles form on the surface of the tissue and may cause it to rise to the surface. This reaction lasts only a short time.

Infiltration with paraffin is as rapid following acetone and dioxane as after chloroform, toluene or xylene. In no instance was infiltration imperfect following these two reagents when they were kept "dry." Infiltration was most difficult after cellosolve. This was perhaps due to water and other impurities since cellosolve is a commercial product and not chemically pure. It is probable that a purer fraction would prove to be more successful in this type of work.

No difficulty was encountered in sectioning after any of the methods, provided, of course, that infiltration was complete. It was thought that sectioning was easiest following dioxane and cellosolve. Acetone often caused a certain amount of brittleness, especially in malignant tissues.

The sections were mounted on the slides with generous amounts of Mayer's albumin after which they were allowed to dry thoroughly. If they are not dry they may come off during the staining process.

Staining.—After the sections are completely dry the slide is placed in xylol until the paraffin is removed. It is not known how much, if any, damage occurs to the tissue while in xylol during this short time. Xylol is the best paraffin solvent that has been tried though dioxane is a fairly good solvent if used hot; but it is not as satisfactory as xylol. Next the slide is transferred to the undiluted dehydrating agent for a minute or more, then into distilled water until the dehydrating fluid is removed. Here, as in all methods, the time for each step may be shortened if the slide is gently moved around to accelerate the exchange between tissue and fluid. Next the slide is stained with Delafield's hematoxylin from which it is transferred to slightly alkaline tap water until sections are blue. Usually no destaining is necessary, but if desired destaining may be

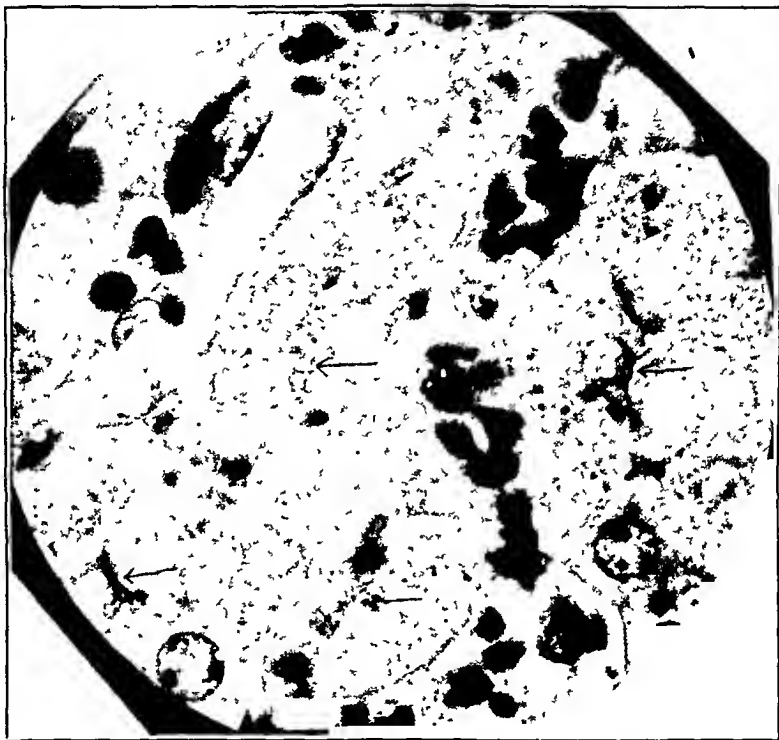


Fig 3.—Human liver. Fixed in 20 per cent formalin and dehydrated in acetone. Iron hematoxylin. Varicosities of bile capillaries can be seen

accomplished in acidified tap water. After tap water it is advisable, but not necessary, to pass the slide through distilled water. At this point the procedure varies slightly for the different reagents used. Aqueous eosin may easily be dissolved in acetone and cellosolve until the desired strength is reached. There, when double staining, both staining and dehydration may be accomplished in the same step. However, it is difficult to dissolve enough eosin in tertiary butyl alcohol and impossible to dissolve any in dioxane for staining purposes. So in the case of the latter two reagents it is necessary to use ordinary aqueous eosin. In all cases it is desirable to wipe the excess water from the slides before placing in the dehydrating agent as this enables them to be used for a longer time. A total of two changes in each of the dehydrating agents is best although one is

often enough. Mounting is done with Canada balsam direct from the dehydrating fluid except in the case of acetone which must be passed through xylol. Acetone is not miscible with balsam and the evaporation is so rapid that the sections dry before mounting is possible. By using the above technique good preparations were made in three to five minutes.

Observations—A piece of tissue was cut into cubes measuring approximately one-half centimeter and each block was treated identically except that each was treated with a different dehydrating agent. Also in all cases a similar block was treated with the usual ethyl alcohol technique. It should be understood that with the exception of the ethyl alcohol no graded series were used.



Fig 4—Human liver. Fixed in Orth's fluid and dehydrated in dioxane. Iron hematoxylin. Bile capillaries showing no varicosities are present. Arrows point to cut ends of bile capillaries.

When the completed slides were compared some differences were apparent. In every case the best slides were obtained by using dioxane or acetone. They were not only better than those which had been dehydrated with cellosolve and tertiary butyl alcohol but were better than those which had been carefully dehydrated with ethyl alcohol and cleared in chloroform. And of course such a technique required only a fraction of the time needed for ethyl alcohol dehydration.

The preparations made using tertiary butyl alcohol and cellosolve were, in most cases, very poor, the cellosolve giving somewhat the better results. However, cellosolve seemed to cause more shrinkage, especially nuclear shrinkage, than any of the other reagents. On the other hand it was most difficult to stain

sections following tertiary butyl alcohol. This was especially true with cancerous tissue, which took a very uneven stain following this fluid. Tertiary butyl alcohol caused a great deal of shrinkage in connective tissue while acetone seemed to cause the least. In fact, the question has arisen, concerning acetone, as to whether it does not even cause a slight swelling of the collagen fibers.

In all of the preparations the cells were more granular than after the use of ethyl alcohol, a result due perhaps to fewer of the cell constituents being dissolved out by these fluids. Because of this the sections often appear more opaque than when ethyl alcohol is used, which may be a disadvantage in some cases. The best preparations of liver were obtained with the use of dioxane following fixation in hot 100 per cent formalin and Orth's fluid. The bile capillaries appeared very distinct and were probably more normal than after acetone treatment where there was some distortion as evidenced by slight contractions and dilations along the capillary.

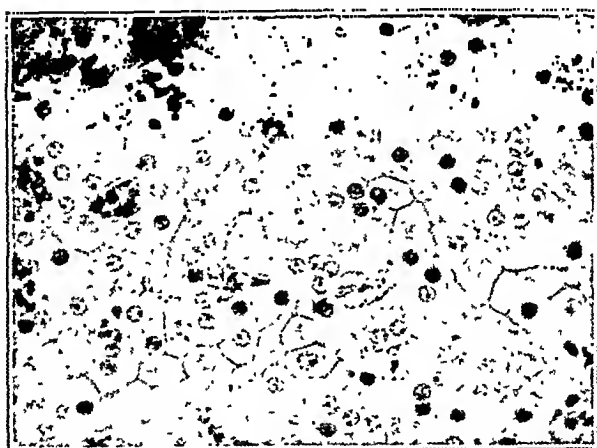


Fig. 5.—Human liver. Fixed in hot 100 per cent formalin and dehydrated in dioxane. Iron hematoxylin. Shows many bile capillaries.

None of the preparations showed anything unusual when examined with polarized light. The striations in the cardiac muscle appeared equally clear following all dehydrating fluids.

Frozen sections were made of some of the tissues and each of the dehydrating fluids was tried on the sections in place of ethyl alcohol. In this material it was found that the best results were obtained with dioxane and tertiary butyl alcohol and that acetone gave the poorest results. This is mentioned, not because any are superior to the ethyl alcohol technique in frozen material but merely to point out that certain of these reagents may be used as a substitute for ethyl alcohol.

It should not be inferred that tertiary butyl alcohol and cellosolve are discredited as dehydrating agents if used differently, perhaps in a graded series. Cellosolve has been used successfully by Frost (1935) on plant tissue. Tertiary butyl alcohol was introduced as a dehydrating agent and used successfully on a variety of materials by Johansen (1935). However, I gather from Johansen's paper that he used a graded series, was interested more in cellular details and was not concerned with the time element.

WARNING

It has recently been reported that the breathing of even a small amount of dioxane fumes causes bronchial, liver and kidney disturbances of a very serious nature. As a result it has been suggested that all work with dioxane be done in a chemical hood. Its use has been forbidden in some British laboratories because of its danger. However, where no hood is available and where the work takes but little time it should be sufficient to work in a well ventilated room and keep the dioxane containers covered at all times when not in use.

SUMMARY

1. A comparative study of four dehydrating agents was made on human tissue following various fixatives. The reagents used were eelsolve, tertiary butyl alcohol, dioxane and acetone. Best results were obtained with the latter two.

2. Briefly summarized into steps the technique is as follows: (1) Fix in hot 100 per cent formalin for five minutes. (2) Wash in running water ten minutes. (3) Dehydrate in two changes of undiluted dehydrating agent for one to one and a half hours. (4) Embed in paraffin. (5) Section. (6) Remove paraffin in xylol. (7) Undiluted dehydrating agent. (8) Distilled water. (9) Stain in hematoxylin. (10) Alkaline tap water until blue. (11) Distilled water. (12) Stain in eosin dissolved in dehydrating agent. (13) One change of dehydrating agent. (14) Mount in Canada balsam. Total time needed from unfixed tissue to completed preparation is about two hours.

It is with pleasure that I acknowledge the following without whose help this work could not have been accomplished: Zoological Laboratory, University of Cincinnati; Charles Goosmann, M.D., Cincinnati Radium Laboratory, and Cincinnati Deaconess Hospital.

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APPARATUS FOR THE EXTRACTION OF ESTRIN FROM URINE*

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THE apparatus† described below was designed for the isolation of estrin from urine. The process is continuous and requires no attention during the period of extraction. The apparatus is inexpensive, rapidly assembled, and sufficiently lacking in rigidity to avoid being fragile. In principle, droplets of chloroform are passed through acidified urine, and the resultant extract flows by gravity into a flask from which the chloroform is evaporated to leave behind the hormone. The vaporized chloroform is subsequently condensed, and passage through the urine repeated.

I. DESCRIPTION OF APPARATUS

Dimensions of the several parts of the apparatus are shown in Fig. 1. All glassware is Pyrex. Flask A is a standard product (ground joint No. 20) into which a safety tube has been inserted. The latter reaches almost to the bottom of the flask and is flared at the outer end. Relatively wide bore glass tubing is employed (11 and 10 mm.), in order to make the apparatus as sturdy as possible. The Liebig type condenser of the size shown, is adequate in effecting condensation of chloroform vapor. Flask B is a stock separatory funnel modified by the attachment of the delivery tube, as shown. The coil in B consists of glass, 1 cm. wide, wound tightly about a glass rod, and supported at the bottom by three prongs. The diameter of the opening in the stopcock shown is 4 mm.

Fig. 2 is a photograph of the assembled apparatus. Flask A rests on an asbestos centered wire gauze. The source of heat is a General Electric hotpoint plate (diameter 6 inches). The 11 mm. tube is steadied with a buret clamp, and connected with the condenser by means of gum tubing. The joint is glass to glass. If the latter precaution is observed, there is no need for a ground glass connection at this point. A contour clamp holds the iron rod which supports the condenser. Flask B rests within an iron ring (inside diameter $4\frac{1}{4}$ inches), and the coil inside is arranged to avoid touching the neck of the vessel. The 10 mm. tube delivers into the flared portion of the safety tube (Flask A). No rubber stoppers or corks are necessary.

II. PROCEDURE

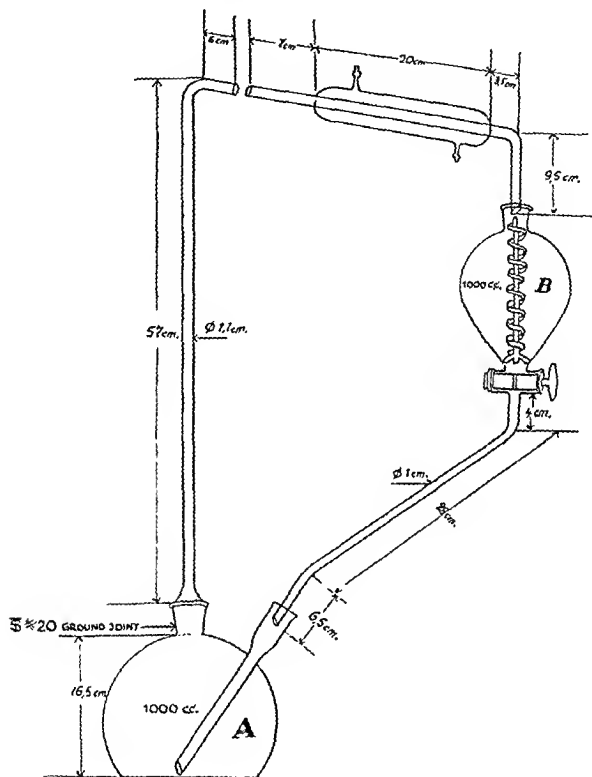
From a twenty-four-hour sample of urine previously adjusted to pH 1.5 (conc. H_2SO_4), 500 c.c. are withdrawn for extraction. The urine is placed in Flask B (stopcock closed), 250 c.c. chloroform added, and the glass coil inserted. In Flask A 500 c.c. chloroform are placed. The apparatus is now assembled as shown in Fig. 2. The condenser is connected with a source of cold water, and

*From the Chemical Laboratory of the Medical Center.

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†Apparatus made by Eck and Krebs, New York, N. Y.

heating begun at *medium*. When chloroform has begun to boil, the plate is turned down to *low*. Delivery of chloroform from the condenser should consist of a continuous series of drops falling upon the central portion of the glass coil.



SCALE 1:4 cm.

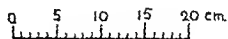


Fig. 1.

The object of the latter is to retard the passage of chloroform through the urine, in order to make the extraction process more efficient. Shortly after distillation has begun, the stopcock on B is opened sufficiently to counterbalance the inflow of chloroform. Extraction is continued for six hours. If not subjected to pro-

longed changes of current, or to a lowering of temperature caused by drafts, the apparatus will continue to operate without need of adjustment during the entire period of extraction.

When extraction is completed, distillation is discontinued, and chloroform in B drained into Flask A. Flask B is removed, the urine discarded, and chloroform in A distilled into a clean, dry container. Recovery of chloroform can be

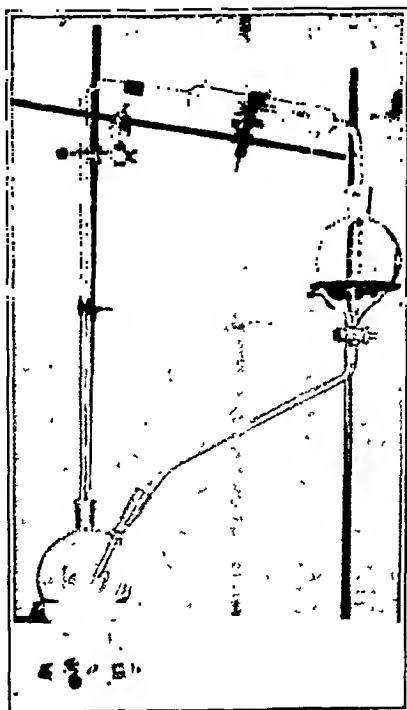


Fig. 2.

carried out with the plate either at *medium* or *high*. The final 100 e.e., however, are boiled off at *low*. Heating is discontinued just before the level of liquid in the flask has sunk below the mouth of the safety tube. The flask is then removed from the plate, cooled under running water, and the contents poured into a dry 50 e.e. beaker. The flask is washed with two 5 e.e. portions of chloroform, and the washings added to the hormone extract. Combined washings and extract may be further concentrated on a water-bath to any desired volume. After the addition of olive oil, the estrin is ready for animal testing.

A NEW CHOCOLATE AGAR FOR CULTURE OF THE GONOCOCCUS*

C H E BECK, MS, PHILADELPHIA, PA

SINCE the discovery of the gonococcus by Neisser, numerous media have been devised and reported for its isolation and culture. Outstanding among these have been the hormone media of Huntoon,¹ Spray's modification² of North's³ medium, and the chocolate agar of Spohn and Landy.⁴ The latter make use of North's gelatin agar (Difco), enriched with sheep blood, which is added with the agar at 75° C, and is prepared just immediately preceding use in order to insure a moist surface.

In a study being conducted on the isolation and cultivation of the gonococcus, all of the usual media proved unsatisfactory for one reason or another. Due to the limited time available and the large amount of material being examined, it was found necessary and desirable to prepare the media in fairly large quantities, sufficient to last several weeks. Considerable difficulty was likewise experienced in getting positive cultures from patients who had only a slight infection or in those who had few gonococci of a weakly growing strain present in the discharge. In the majority of these cases the contaminating organisms rapidly overgrew the gonococci before isolation or identification could be effected.

A medium was sought which would enhance the growth of these weakly growing or slow developing strains, allowing a more rapid isolation and identification in pure culture.

The medium finally selected as most nearly meeting the requirements was prepared as follows:

Take 77 grams of Bacto North Gelatin Agar (Spray's formula) and dissolve in 1,000 c.c. of distilled water by boiling several minutes. The medium thus prepared is dispensed in 1,000 c.c. Erlenmeyer flasks, 500 c.c. in each flask. The flasks are stoppered with gauze and cotton plugs, capped with heavy brown Kraft paper and autoclaved at 20 pounds for thirty to fifty minutes. Flasks are cooled to 55° to 60° C, 50 c.c. of defatted beef blood added to each, and gently rotated to insure thorough mixing of blood and agar. At this temperature an even distribution of blood throughout the agar is easily obtained, whereas at higher temperatures the blood tends to coagulate before it can be thoroughly distributed, causing lumps in the final medium. The flasks are then placed in a water bath at approximately 60° to 65° C, and the temperature gradually raised to 100° C. About fifteen to twenty minutes should be consumed in raising to this temperature. The blood and agar mixture must be frequently agitated by gently rotating the flask, during the entire heating process care being exercised to prevent foaming. Hold at 100° C for twenty minutes. At the end of the heating period, the

*From the Laboratories of John Wyeth & Brother, Inc.
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agar is rapidly cooled to about 55° C. and poured into petri dishes, 15 to 20 c.c. each. Cooling below this temperature makes the agar extremely difficult to pour, as it congeals at about 45° C.

The advantages of this medium over others used are numerous, outstanding among which are:

1. Beef blood is used, which can be obtained at any slaughter house. It need not necessarily be collected aseptically, only the usual precautions of cleanliness being observed. The heating process is sufficient to kill all ordinary contaminants. All plates are given a preliminary incubation before use, and no contaminants have been observed on any plates before inoculation.

2. Petri dishes may be prepared well in advance of the time they are to be used. Plates prepared and kept at ice box temperature four to five weeks were found to give results equally as satisfactory as freshly prepared ones.

3. Excellent growth of even the more weakly growing strains is obtained in twenty-four hours, and in many cases, colonies of the gonococcus have been observed before any of the contaminants were visible.

4. A higher percentage of positive results was made possible on borderline cases with this medium.

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A NOTE ON THE STAINING OF TUBERCLE BACILLI IN SECTIONS*

DOROTHEA SCHMIDT FULLER, M.A., New York, N. Y.

IT IS commonly believed that fixation of tissues in formalin renders them unsuitable for staining acid-fast bacteria. A method for staining tubercle bacilli which gives satisfactory results after formalin and after Zenker-formol seems, therefore, worth recording. The method which follows yields good staining of the bacteria with either of these fixatives. With formalin-fixed tissue the green counterstain is prone to fade in a few months, the bacteria and nuclei remaining well stained indefinitely. With tissue fixed in Zenker-formol, all structures retain the dyes for prolonged periods.

In general the method consists of staining in iron hematoxylin, followed by fuchsin, and employing light green as a counterstain. This gives a clear staining of the nuclei; the nuclear membrane is dark blue green and the nucleoli are blue green, with a paler light green for the cytoplasm against which the red bacilli show up brilliantly.

*From the Laboratories of the Rockefeller Institute for Medical Research, New York.
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PROCEDURE

- 1 Run slides down to water as usual and rinse in distilled water
- 2 Mordant in iron alum solution in the oven for five minutes (45° to 50° C)
- 3 Rinse at the tap
- 4 Stain in hematoxylin, also in the oven, for five minutes
- 5 Put directly into the picric acid for five minutes, or longer if necessary, until the stain remains only in the nuclei
- 6 Wash thoroughly in running water (fifteen minutes or longer) until the sections are thoroughly free of the picric acid
- 7 Place on the heating bar and pour the fuchsin solution over them. Heat until steam comes off, but do not boil. Let cool for three minutes, heat again, and again allow to cool for three minutes
- 8 Destain in acid alcohol for a few seconds, until the fuchsin starts coming off in clouds, rinse in tap water and continue destaining until the sections are barely pink
- 9 Rinse in water and put into alkaline water for a few seconds
- 10 Wash thoroughly in running water for ten minutes or more
- 11 Stain in the light green solution for five minutes
- 12 Rinse in water and run up quickly through the alcohols and xylol, mount as usual

SOLUTIONS

- 1 *Iron alum*—5 per cent aqueous solution of iron alum
- 2 *Hematoxylin*—Dissolve 1 gm of Grubler's hematoxylin in 80 cc of hot distilled water cool and add 10 cc of glycerin and 10 cc of 95 per cent alcohol
- 3 *Picric acid*—Add 2 parts of a saturated alcoholic (95 per cent) solution of picric acid to 1 part of 95 per cent alcohol
- 4 *Fuchsin*—Add 16 cc of a saturated alcoholic (95 per cent) solution of fuchsin to 84 cc of aniline water
- 5 *Acid alcohol*—3 per cent nitric acid in 95 per cent alcohol
- 6 *Alkaline water*—Add a little ammonia to distilled water
- 7 *Light green*—1 per cent aqueous solution of light green (Grubler)

DISCUSSION

This method has been used over a period of three years and has been applied to tuberculous tissues from several animal species inoculated with various strains of tubercle bacilli. Aside from technical facility, it has certain other distinct advantages. It gives good nuclear and cytoplasmic staining, so that fine cellular differentiation is obtained. This is particularly evident in such tissues as bone marrow, in which sections so stained are useful not only for studies of tubercle bacilli but also for studies of the marrow cells.

The method is excellent for purposes of microphotography, as tubercle bacilli are stained brilliantly red while nuclei and cytoplasm take different values of green so that excellent contrast is obtainable. For ordinary microscopic examinations, the red green contrast has been found superior to other staining methods.

By this method tubercle bacilli are equally well stained in sections from all viscera. Whereas certain other methods have been found unsatisfactory for staining these organisms in brain and meninges, the technique described herein has been found highly satisfactory in a large series of animals subjected to intracerebral inoculation. Intracellular organisms, so often appearing purple or black when stained by other methods, are brilliant red in this method and are, therefore, easily distinguishable from nonacid-fast bacteria or nonbacillary material. There has been no evidence that bacteria other than tubercle bacilli retain the fuchsin; moreover, distinctive characteristics of individual bacteria, such as beading, are well maintained.

THE GRAPHIC REPRESENTATION OF THE BLOOD PICTURE

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SINCE the significance of the leucocyte count depends upon a number of factors, the ideal way of expressing it would be in the form of a curve, provided the number of factors to be expressed were not too great. Such a curve would be valuable in showing at a glance the significance of the leucocyte count, and in that it would enable the blood picture during the course of an illness to be charted. By means of the method herein described, a curve may be constructed, which will express the severity of the infection, the degree of resistance, and the number of immature neutrophils, both in a qualitative and quantitative manner. It is based upon the Gibson Chart, but expresses, in addition, the number of immature neutrophils, both qualitatively and quantitatively. In our opinion, any chart or graph of leucocytes which fails to include this factor is inadequate.

While in the early stages of an acute pyogenic process, there may be no significant changes in the number of immature neutrophils, there are times when it is the chief, if not the sole factor of any value, in determining the prognosis. Nicholson states that the immature-mature count taken at four- to eight-hour intervals is a better guide to the patient's condition than the temperature chart.

METHOD

The curve to be described is not dissimilar to that which has been devised to express the significance of the blood sedimentation rate. As in the case of the sedimentation curve, a curve of one degree of slope means one thing, a curve of less slope, another, and a straight line bears still another significance. The principle of the Gibson Chart has been taken as the basis for constructing the curves. A horizontal curve (i.e., a curve whose ends are on a horizontal line) indicates that the resistance equals the infection. Curves sloping downward to the right indicate in a general way that the infection is less than the resistance; while curves sloping upward to the right suggest that the infection is in excess

of the resistance. According to Neal, if the neutrophile percentage is above the white count to the extent of five divisions in surgical cases, it is an indication for operative intervention.

To express the degree of increase in immature neutrophils, an arbitrary scale has been chosen corresponding to the divisions on the chart. The percentage of immature neutrophils is given by the distance of the midpoint on a

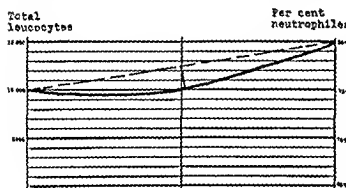


Chart I



Chart III



Chart V

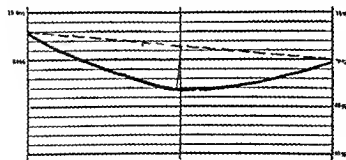


Chart VII

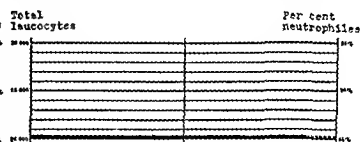


Chart II

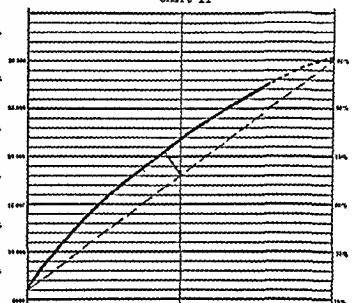


Chart IV

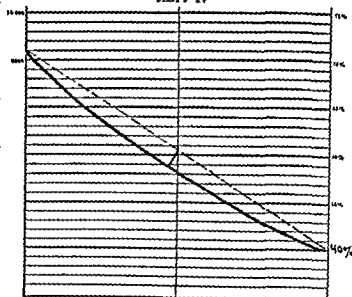


Chart VI

Charts I to VII.—The curve of the blood picture in various diseases. I.—A typical severe bronchitis; total leucocytes 10,000; per cent neutrophiles 80; staff cells 35; segmented neutrophils 20,000; per cent neutrophiles 85; staff cells 40; 35. III.—Fatal pneumonia; total leucocytes 20,000; segmented neutrophils 15; myelocytes 5; segmented neutrophils 10; per cent neutrophiles 15. V.—Cent neutrophils 80; staff cells 60; metamyelocytes 10; myelocytes 1; segmented neutrophils 8. VI.—Malaria fever; total leucocytes 6,000; per cent neutrophiles 40; staff cells 30; segmented neutrophils 10. VII.—Normal leucocyte count; total leucocytes 8,000; per cent neutrophiles 70; staff cells 5; segmented neutrophils 65. (Dotted portion of curve represents red; solid portion represents blue.)

curve, drawn from the figure for the total number of leucocytes on the left to the neutrophile percentage on the right, from a dotted line joining the same points. Each division on the chart represents an increase of 10 per cent in the number of immature neutrophiles. Myelocytes, metamyelocytes, and staff cells are included in the immature neutrophiles in determining the distance of the curve from the dotted line. All curves above the dotted line represent percentages above fifty, and curves below the dotted line represent percentages below fifty. A curve with a distance from its midpoint to the dotted line of five divisions, below the dotted line, represents a zero percentage of immature neutrophiles. A distance of two and one-half divisions represents a percentage of twenty-five, etc. When the curve and the dotted line coincide, the immature percentage is equal to fifty. According to Osgood, a staff count above 50 per cent usually indicates a fatal outcome; therefore, all curves above the dotted line may be said to suggest a fatal outcome.

In order to express the increase in immature neutrophiles qualitatively as well as quantitatively, it is necessary to construct the curve of two different colors. Thus, a count composed of 25 per cent juveniles (metamyelocytes) may be represented by drawing one-fourth of the curve in red, and the remainder in blue. In this way, one can tell at a glance whether the shift is composed entirely of staff cells (degenerative) or if it contains juveniles as well (regenerative), and, in addition, can perceive the relative amounts. Also, when a number of charts are made throughout the course of an illness, the point at which juveniles and other less mature forms first begin to appear, or "the first danger signal" in some cases of infection, can be detected.

To construct the curves with the least effort, a square shaped like a "T" should be used. The vertical line in the center of the chart gives the midpoint of any line drawn across the chart. By placing the base of the "T" across the chart, the dotted line connecting the figure for the white count with the neutrophile percentage can be drawn. Then the perpendicular can be drawn from the center of the dotted line along the leg of the "T," which has been marked off in divisions corresponding to those on the chart. The curve is then drawn by connecting the two ends with the midpoint, located at the proper distance from the dotted line along the leg of the "T." To construct the curve in colors, a red and blue pencil may be used, such as may be purchased at any printer's supply house.

DISCUSSION

When this method has been applied in a number of infectious diseases and pathologic states, it is seen that several different curves with rather characteristic forms result, enabling the hematologist at once to place the curve in one of the various classes. There is a conspicuous difference between the "infectious fever type" of curve with good resistance, and a moderate increase in immature neutrophiles (as in Malta fever), and the "toxic type" (as in pneumonia), where the increase in immature neutrophiles is very marked, and the infection is greatly out of proportion to the patient's resistance. Various other forms of the curve are seen in appendicitis and other acute abdominal conditions. In infections of a more or less minor nature, the curve approaches the normal type.

In our consideration of this subject, we at first thought of using the immature mature ratio, or "filament nonfilament count," as it is sometimes called, as the basis for constructing the curve of the immature forms. This is still taught in some medical schools and in some textbooks as the method for determining the prognosis from the "shift to the left." However, it was found that the immature mature ratio varies to such an extent with changes in the percentage of total neutrophiles, that its value in prognosis is questionable. For instance, it is possible for the immature forms to be considerably in excess of the mature, while the patient is in no way dangerously ill. This naturally follows, since as the number of total neutrophiles decreases, the smaller the degree of increase in the immature forms necessary to make a marked change in the immature mature ratio. *The physician, therefore, should not follow the rule that is occasionally used, that when the immature forms have increased to the point where they are equal to, or in excess of the mature forms, the prognosis should be considered grave.* If one wishes to use the immature mature ratio in prognosis, it is necessary to use the rule given by Osgood, that 50 per cent staff cells suggest a fatal outcome, and to determine the significance of the immature mature ratio at each change in the neutrophile percentage. This may be tabulated somewhat as follows:

PERCENTAGE OF TOTAL NEUTROPHILES	MINIMUM IMMATURE MATURE RATIO INDICATING A FATAL OUTCOME
100%	1 1
95%	1 11 1
90%	1 25 1
80%	1 66 1
70%	2 50 1
60%	5 00 1
55%	10 00 1
50%	100% Immature

It would appear, therefore, that below 50 per cent total neutrophiles, it would be impossible for any immature mature ratio to indicate a fatal outcome. Yet, in spite of this fact, and the fact that the significance of the immature mature ratio depends almost wholly upon the percentage of total neutrophiles, the following may be found in a current textbook, frequently used by medical students and technicians:

IMMATURE	MATURE	SIGNIFICANCE
1	5	Lower limit of normal for mature forms
1	3	Definite infection
1	1	Severe infection
1+	1	Frequently fatal, if persists

It seems far better to us to use some standard, which remains the same under all conditions. The interpretation given by Osgood, which we have incorporated in our method for constructing the curve of the neutrophile changes, we consider much superior to the above. This is based upon the absolute increase in the number of staff cells, over 10 per cent indicating a severe infection, over 25 per cent, a very severe infection, and over 50 per cent a fatal outcome. It furnishes a fixed standard, which remains unchanged regardless of the fluctuations in the other factors in the differential count.

The slope of the line or curve is of great importance. A large number of immature neutrophils has a much more serious import if the neutrophile percentage is greater than can be accounted for by the mere elevation of the total white count. This is also true if the increase in immature neutrophils contains metamyelocytes and myelocytes. A "regenerative shift" is always of graver prognostic significance than a "degenerative shift."

Thus, by the use of the chart, one is enabled to grasp all the various complementary factors in the differential count at a glance. The presence and approximate amount of metamyelocytes and myelocytes, the degree of increase in immature neutrophils, the percentage of total neutrophils, and the extent of the patient's resistance in relation to all these factors, can all be portrayed in a few seconds by the construction of a simple curve; and, therefore, it is evident that an entirely different set of principles for appraising the prognosis from those which could be expressed by the original Gibson Chart can be laid down.

It is well to bear in mind the chief exceptions to the rules which have been made concerning increases in staff cells and metamyelocytes. Malaria is given as the chief exception to the rule that over 50 per cent staff cells indicates a fatal outcome. There is also a rule in regard to metamyelocytes, that the presence of more than 5 per cent in the blood indicates a seriously ill patient. The exceptions given are malaria, osteomyelitis, and smallpox. We are inclined to think that diseases ordinarily characterized by the presence of myelocytes and metamyelocytes in the blood, but without a very high mortality, of which the typhus fever seen in this country is a good example, also belong in this class. Schilling speaks of "the gay blood picture" which characterizes typhus, by which he means the leucocytosis, the high staff count, and the myelocytes and metamyelocytes, which are usually seen.

SUMMARY

A method for expressing the essential elements of the leucocyte count in the form of a curve is described. It presents the following new features:

1. It expresses the increase in immature neutrophils (or "shift to the left"), both qualitatively and quantitatively, in addition to the total white cell count and neutrophile percentage.

2. When the method is applied to the interpretation of the leucocyte count in a number of pathologic states, various forms or "types" of curves result, which enables a given count to be at once placed within one of the different classes.

3. It simplifies the interpretation of the leucocyte count from the standpoint of prognosis.

4. It makes it possible to record the entire blood picture in the form of a chart during the course of an illness, showing the point at which metamyelocytes and myelocytes first appear in the blood, the rate and degree of increase in the total immature neutrophils, and the extent of the patient's resistance in relation to the progress of the infection.

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AN ANALYSIS OF COLORIMETRIC METHODS IN RELATION TO PLASMA VOLUME DETERMINATIONS

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A FEW years ago the author found that certain bodily states which are attended by thirst and a reduced salivary flow are also associated with a decrease in the plasma volume (Gregersen, 1932, Gregersen and Bullock, 1933). In the course of these experiments, however, it soon became evident that repeated determinations of plasma volume with the method devised by Keith, Rowntree and Geraghty (1915) were of questionable accuracy, even when the modifications suggested by Smith (1920) were followed. The author therefore decided to abandon the original investigation until a reliable technique for repeated determinations could be developed. With this purpose in mind the dye method has been examined in detail and the assumptions on which it is based have, so far as possible, been tested experimentally. The final reports have been withheld for nearly three years in order to try out the technique in a variety of physiologic problems; the experience gained has above all been valuable in demonstrating the limitations of any dilution method for estimating plasma volume. In view of the growing interest in the study of the water balance, it now seems desirable to report briefly on some of the more important questions which have been considered. The intention has not been merely to prepare a guide for those wishing to use the dye method but to provide a basis for evaluating the mass of blood volume data which has accumulated since Keith, Rowntree, and Geraghty introduced the plasma dye method in 1915. Nevertheless, it is important to remember that so many conflicting results have been obtained with the plasma dye method during the past twenty years that the testing of the method itself has in reality become the cardinal issue. First to be considered is the problem of colorimetry. The difficulties encountered and ways of overcoming them may be illustrated by discussing only three instruments, the simple colorimeter, the compensating colorimeter, and the spectrophotometer.

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The Simple Colorimeter.—The ordinary colorimeter is not satisfactory for accurate work unless the solvent itself is entirely clear and colorless. Such an ideal condition is, of course, never approached in plasma. To cancel the natural plasma color Keith, Rowntree, and Geraghty (1915) made up the standard and unknown in the same dilution of plasma constituents. Even so, it must be obvious that the contribution which the plasma itself makes to the total color effect is not the same in the standard and unknown solutions unless the solutions are the same depth. True cancellation of the natural plasma color is therefore not obtained unless the dye concentration happens to be exactly the same in both.

An examination of the literature reveals that these facts have not been universally recognized and it will be seen presently that a failure to do so may lead to serious errors, especially when the plasma contains a residuum of dye from a previous injection. Under this circumstance the plasma volume must be calculated from the *increase* in plasma-dye concentration resulting from the injection of a known quantity of a standard dye solution. Two methods for measuring this *increase* in dye concentration with the simple colorimeter have been worked out by Smith (1920; 1925), but each involves two separate colorimetric determinations and a number of laborious manipulations. Although Smith's methods do not seem to be practicable (Miller and Poindexter, 1932), his analysis of the problem makes it possible to account for some of the paradoxical and conflicting results which have been obtained with repeated determinations (Robertson and Boek, 1919; Lamson and Rosenthal, 1923).

The conditions which limit the use of the simple colorimeter and the errors incurred by ignoring them may be seen from the equation

$$\frac{R_2}{100} = \frac{R_1 + R_3}{R_1 + 100} \text{ (Smith, 1920).}$$

" R_2 = observed colorimetric reading (expressed in per cent) of a sample of dye-colored plasma taken after the injection of dye . . . against a standard prepared from dye-tinged plasma taken immediately before injection of dye. . . . R_1 = the observed colorimeter reading of a sample of dye-tinged plasma . . . taken immediately before the injection of the dye . . . read against a standard prepared . . . (from) normal dye-free plasma. . . . R_3 represents the increase in concentration of dye in the plasma as a result of the injection." As Smith states, "this formula simply means that the unknown ($R_1 + R_3$) divided by the standard ($R_1 + 100$) equals the observed colorimetric reading in parts per 100 (i.e., R_2)."

From the above equation, we may conclude:

- (1) $R_2 = R_3$ when $R_1 = 0$ (no residual dye).
- (2) If $R_2 = 100$, then $R_3 = 100$ at any value of R_1 (i.e., when standard and unknown are the same strength no error results from the presence of residual dye).
- (3) When $R_2 > 100$, $R_3 > R_2$ (i.e., when the standard is weaker than the unknown, the observed dye concentration is too low and the calculated plasma volume correspondingly high).
- (4) Conversely, when $R_2 < 100$, $R_3 < R_2$ (i.e., when the standard is stronger than the unknown, the observed dye concentration is higher than the true value and the calculated volume accordingly too low).
- (5) Any difference between R_1 and R_3 increases with R_1 .

The degree to which residual dye (R_1) may affect the measurement of plasma volume when the observed colorimetric reading (R_2) is not equal to 100 per cent is clearly demonstrated by the following experiment.

The plasma volume of a normal dog was determined four times in about three hours. In the initial determination the concentration of the standard (1/300) was adjusted to equal that of the unknown ($R_2 = 100$ per cent). In the succeeding determinations four standards (1/200, 1/250, 1/300 and 1/400) were prepared each time from the dye-tinged control plasma obtained immediately before the injection of dye. It may be seen in Fig. 1 that except with the 1/300 standards against which the unknowns gave colorimetric readings of 100 per cent the results did not check with the original determination. The magnitude and direction of the error is obviously determined by the relative concentrations of the standard and unknown. If the standard is stronger than the unknown, the estimated volume is too low; if it is weaker than the unknown, the reverse is true. The range of error is apparently so large that extensive changes in plasma volume might readily be missed with such a method. It is hardly necessary to point out that lipemia and to a lesser degree

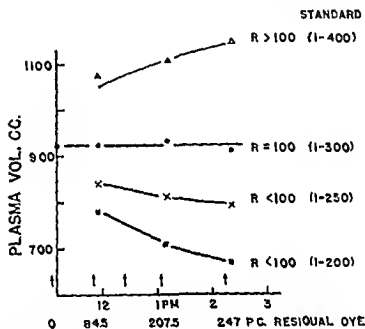


Fig. 1—Repeated determinations of plasma volume with the Keith-Rowntree method showing the results obtained by reading each unknown against different standards. June 19, 1933. Dog 20.6 kg. Injections of 4 cc. of 1.0 per cent brilliant vital red marked by arrows. The third determination was not completed due to hemolysis of the samples. For discussion of experiment see text.

the natural plasma color may also invalidate the results, and therefore the unknown should in every case be read against a standard which matches it very closely.

The Compensating Colorimeter.—Colorimeters that incorporate the principle of the compensating block comparator, well-known in connection with colorimetric pH determinations on tinted fluids such as urine or plasma (Clark, 1928), have in recent years been widely used for determinations of plasma volume (Seyderhelm and Lampe, 1925; Lindhard, 1926; Fleischer-Hansen, 1929-1930; Rusznyak, 1927; and others). A schematic diagram of one of these instruments, the Ellinger modification of the Bürker (1924) colorimeter is shown in Fig. 2. The cups are fixed, and the two plungers on each stage can only be moved together; hence the light beams *A* and *B* always traverse equal depths of fluid. With the arrangement of the solutions indicated in Fig. 2 the color contributed by the solvents is automatically cancelled, regardless of the

relative concentrations of dye in the standard and unknown and of other differences in their composition. For this reason a number of investigators have been led to simplify their technic by using dye standards prepared in water (Lindhard, 1926; Fleischer-Hansen, 1929, 1930; Griesbach, 1921, 1928; Schmidt, 1927; and others). Such a practice is not permissible with many vital dyes because their spectral absorption curves in water and plasma are quite different (Gregersen and Gibson, 1937).

The compensating colorimeter should also eliminate the difficulties with residual dye. If a portion of the dye in the unknown is residual, the same

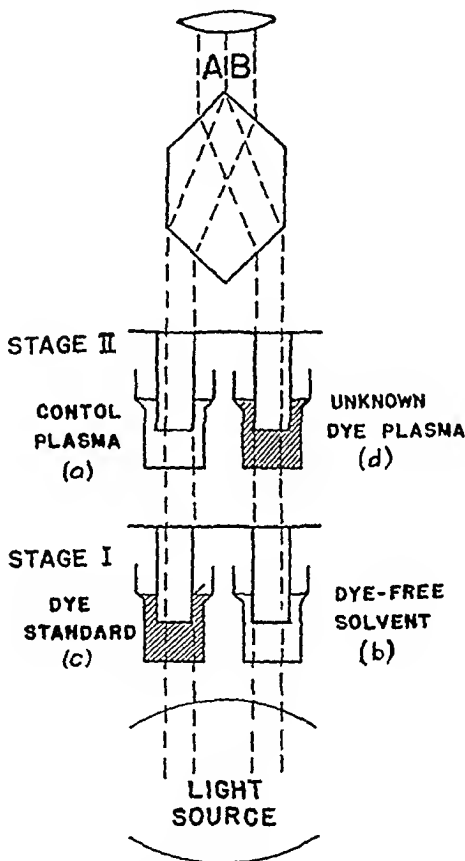


Fig. 2.—Diagram of the Ellinger modification of the Bürker colorimeter. (After Freund, 1932.)

concentration of residual dye will, of course, be present in the control sample. Under these conditions the dye standard in *a* (Fig. 2) is not compared with the total dye concentration in *d* but only with the difference in dye concentration between *c* and *d*, i.e., with the increase in color obtained as the result of the injection. Hence, a second or third determination of plasma volume should be no more complicated than the first in which the control plasma is actually free from dye.

In spite of its theoretical advantages, the compensating colorimeter proved to be very unsatisfactory. Most disturbing was the observation that in the analysis of a plasma dye sample the ratios obtained with the standard adjusted

to various depths sometimes showed differences of 30 to 40 per cent. The cause of this was not discovered. Furthermore, if the samples showed any lipemia the results were quite unpredictable, a fact also reported by Lindhard (1926). Known solutions were tested against themselves in an attempt to study the nature of the error caused by lipemia, but the only conclusion which could be drawn was that the readings fell nearer the correct values if comparisons were made with fluid layers 5 mm. or less in depth. Curiously enough, a series of known solutions of brilliant vital red made up in diluted milk gave correct readings although the scattering of light in these solutions was intense.

Practical difficulties appear also when the blood samples are diluted with isotonic oxalate to prevent clotting (Hooper, Smith, Belt and Whipple, 1920). Since the control and unknown must be identical in every respect except for the dye concentration, it is obvious that analysis with the Burkner colorimeter is not valid unless the plasma dilution is exactly the same in both samples. This is especially true when the plasma contains residual dye. Inasmuch as the oxalate must be added to the whole blood, the degree of dilution is always a matter of chance.

Finally, there is the particularly troublesome problem of hemolysis. The red dyes which have been commonly used for determining plasma volume (brilliant vital red, congo red, vital red, or trypan red) match the hemoglobin so closely that hemolysis of a dye-tinged sample may escape notice and give a totally erroneous reading for the concentration of the dye. The analysis is quite impossible if either the control or the unknown is hemolyzed. Consequently it is necessary to discard all samples which show even faint hemoglobin bands spectroscopically (Griesbach, 1921, 1928, Lindhard, 1926).

The Spectrophotometer. *—This instrument offers a solution for the difficulties which are encountered with the colorimeters. Not only is the accuracy of measurement (1 per cent or less) far greater than with the colorimeters, but the determinations may be made on smaller samples (0.5 to 1.0 c.c.). Since the color is measured in absolute terms (optical density), the spectrophotometer eliminates the necessity of preparing standard solutions at the time of analysis of the unknown. Ordinarily the natural plasma color or residual dye is directly cancelled by reading the unknown against the control in a pair of identical absorption cells. If this is impossible due to unequal oxalate dilutions the optical density of each sample can always be read separately, and appropriate corrections made for the differences in dilution.

Lipemia, which makes it impossible to employ the compensating colorimeter, does not interfere with the correct determination of the dye concentration with the spectrophotometer, provided the control shows the same degree of opalescence as the unknown. This was demonstrated in the following experiment. Two samples of plasma, one clear and the other showing intense lipemia (one hour after a fatty meal), were taken from the same dog and mixed in various proportions. Two tenths of a cubic centimeter of a standard dye solution (1/50 of 1 per cent T 1824) were then added to 1.8 c.c.

*For description, theory and use of spectrophotometers the reader is referred to Clark (1928), H. P. Smith (1930) and also to *The Practice of Spectroscopy* by Twyman and Allsopp (1934) which, although it deals with only one make of instrument, contains information that applies to all types of spectrophotometers.

of each mixture, while control (dye-free) solutions were prepared by adding 0.2 c.c. of saline to another 1.8 c.c. portion of each. When the samples were read in the spectrophotometer against their corresponding dye-free controls, the optical densities were found to be the same for all. The presence of fat does not, therefore, alter the optical density of the dye.

Nevertheless, lipemia cannot be ignored in carrying out plasma volume determinations. Any alteration in the intensity of the lipemia between the taking of the control sample and any subsequent dye sample will, of course, introduce an error in the determination. This is especially disturbing in studies on the disappearance rate of dyes from the blood stream, in which case it must be tacitly assumed that no change has occurred in the optical density of the plasma itself during the experiment. Lipemia can ordinarily be avoided by using only fasting animals or subjects, but in those cases in which it is an inescapable feature (e.g., myxedema), the plasma samples should be washed with a fat solvent before spectrophotometric analysis is attempted. Ethylene chloride is the best clearing agent tried so far, but it is not entirely satisfactory. After this treatment the samples are still somewhat opalescent.

The great advantage of spectrophotometric analysis is best seen in dealing with hemolysis. Whereas the error from this source cannot be avoided with a colorimeter, the spectrophotometer offers two possible ways of coping with the problem.

1. It is quite apparent from Fig. 3 that a simple spectrophotometric determination in plasma of a red dye such as brilliant vital red is out of the question if the sample is so much as slightly hemolyzed. Nevertheless, the spectral absorption curve of this dye differs sufficiently from that of hemoglobin to make possible a correction for hemolysis (Graff and Clarke, 1931). The method is in principle the same as that described by H. P. Smith (1930) for the analysis of a mixture of brilliant vital red and niagara sky blue, and depends upon readings taken at two points of the spectrum where one substance shows maximal and the other nearly minimal absorption. This procedure is time consuming and involved and, if the control sample also happens to be hemolyzed, it is impossible to determine accurately the absorption due to the natural plasma color. For routine experiments the method cannot be recommended.

2. Corrections for hemolysis become practically unnecessary by the choice of an appropriate dye. It may be seen from Fig. 3 that the spectral absorption of hemoglobin falls off sharply between 580 and 600 μ ., and that in the region from 610 to 700 μ . it is relatively insignificant. Blue dyes which have maximal absorption in this part of the spectrum could, therefore, be determined directly in hemolyzed samples without much error; but the majority of blue dyes leave the blood stream so rapidly that they cannot be used for determining the plasma volume. However, among those studied by Dawson, Evans, and Whipple (1920) and by Gregersen, Gibson, and Stead (1935), there was one, T-1824, which disappeared from the plasma at a very slow rate (5 to 10 per cent per hour). Since the spectral absorption peak of T-1824 in plasma (Fig. 3) falls at 620 to 625 μ ., a considerable degree of hemolysis

is required to interfere seriously with the direct determination of this dye. It should also be pointed out that the cancellation of the absorption due to the plasma itself assumes a less important rôle with blue than with red dyes, since the absorption curve of plasma is considerably lower at and beyond 600 mμ than it is at 500 mμ.

Other investigators have recommended the use of blue dyes for plasma volume determinations (Dawson, Evans and Whipple, 1920, Harris, 1920, Seydelhelm and Lampe, 1922, and others) but only because blue seems rather easier to read in a colorimeter and does not obscure hemolysis. In my experience, however, a blue dye presents no great advantage unless the analysis is carried out with a spectrophotometer. In fact, it has the distinct disadvantage that the amount which must be injected (1 mg per kilogram) to yield a

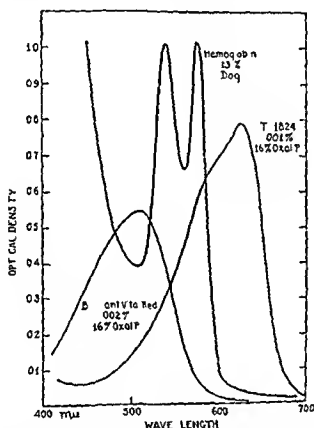


Fig. 3—Spectral absorption curves (Hardy's self recording spectrophotometer) of brilliant vital red hemoglobin and the blue dye T 1824 showing that spectrophotometric determinations of the latter in plasma are affected relatively little by the presence of moderate amounts of hemoglobin whereas in the case of red dyes such as brilliant vital red hemolysis introduces a large error.

plasma concentration that can be read conveniently in a colorimeter causes marked staining of the skin and sclera and gives the subject an unhealthy, cyanotic appearance. It was for this reason that Seydelhelm and Lampe (1925) abandoned the trypan blue method which they had previously advocated (1922). If, on the other hand, the dye determinations are made with a spectrophotometer equipped with 20 mm absorption cells it is possible, without sacrificing accuracy, to use such small doses of dye that staining is practically eliminated. T 1824, for example, can be measured accurately in concentrations ranging from 0.001 to 0.0005 per cent. Therefore, 20 mg of this dye are sufficient for a plasma volume determination in man. This amount does not produce visible staining.

CONCLUSIONS

1. The use of the simple colorimeter for determining plasma volume with the dye method may involve gross errors, especially when the plasma contains residual dye (Smith, 1920). The result obtained depends very largely upon the relation between the strength of the standard and the unknown (Fig. 1).

2. The compensating colorimeter fails to yield dependable results under conditions which appear to be suitable for estimating the plasma-dye concentration with this type of instrument. Furthermore, if the samples are turbid (lipemia) or hemolyzed, the compensating colorimeter cannot be employed at all (Lindhard, 1926).

3. These difficulties can be overcome by using a spectrophotometer. With this instrument the absorption due to natural plasma color, lipemia or residual dye is directly cancelled by reading the unknown (dye sample) against the control (dye-free sample); a second or third determination may therefore be carried out as accurately and as readily as the initial one.

4. It is evident from the spectral absorption curves in Fig. 3 that the tedious corrections for hemolysis necessary with red dyes (Graff and Clarke, 1931) can be eliminated, except in grossly hemolyzed samples, by using the blue dye T-1824.

The author wishes especially to acknowledge his indebtedness to Dr. W. B. Cannon who provided the costly equipment required for this investigation. It is also a pleasure to thank Dr. H. M. Evans of the University of California for the supply of T-1824 with which many of the preliminary experiments were carried out, and Dr. A. C. Hardy of the Massachusetts Institute of Technology for the use of his self-recording spectrophotometer.

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CORRELATION OF HISTOLOGIC STRUCTURE WITH CLINICAL FEATURES*

EXEMPLIFIED BY LABORATORY REPORTS ON GOITERS

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IT IS a commonplace for one and the same histologic section of an enlarged thyroid to show several kinds of change, whatever the clinical picture may be. In consequence, both surgeon and physician are likely to assign less importance to the laboratory report than they would if each type of thyroid enlargement carried its own distinctive picture. On the other hand, there may be no evident deviation from the normal structure at all, in which case the surgeon is not willing to conclude that his thyroidectomy was superfluous. This lack of correlation between histologic structure and clinical features has also naturally led to a distinctive nomenclature on the part of surgeon, physician, and pathologist. To some extent this could be obviated by greater attention to detail in the recording of the microscopic characters, for, if the various kinds of change found in a given specimen were listed, and at least a relative numerical value assigned to each, a tangible composite could be offered the clinician, from which he could recognize correspondence with his own observations.

The normal thyroid gland is of course a *tout ensemble* of a number of smaller functional units. These in turn are composed of various simpler components: cells, vessels, nerves, connective tissue. The diseased gland shows modifications of these, and in addition other features belonging to general pathology.

Since the standard textbooks on histology are necessary laboratory equipment, it is superfluous to do more than enumerate the following component elements in the thyroid acinus unit: (1) secretory cells, comprising some six varieties (including the so called "parafollicular cells") in each of which one considers (a) mitochondria, (b) Golgi apparatus, (c) granules, (2) the colloid, (3) the capillaries and lymphatics, (4) the sympathetic nerve fibers, (5) the connective tissue elements common to any organ. In the first place according to the functional state of the gland, we note in the sections "R," the resting or involution phase of the acinus (acini or "vesicles" fairly large, the contained colloid staining fairly deeply with eosin, lining cells cubical or flattened), and/or "H," the hyperactive acinus (acini smaller, poorly staining colloid, lining cells tending to columnar form). The same symbol "H" may stand for "hypertrophy" and also for "hyperplasia," in which condition all the acini show hyperactivity up to an actual over production of cells. "I" denotes "islets" of the so called parafollicular cells, external to the acinus itself.

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Second, in pathologic states we notice aggregations of lymphocytes "l," varying from a few cells in number to collections big enough to call "lymphomas"; "h" stands for haemorrhages, which may occur into or between the acini; "f" stands for the fibrous stroma when abnormal in amount; calc = calcification. We also allow for collections of other inflammatory cells, collections of secretory cells of parathyroid type, collections of secretory cells mimick-

Fig. 1.

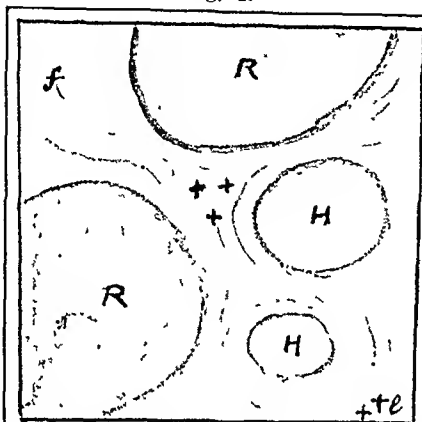


Fig. 2.

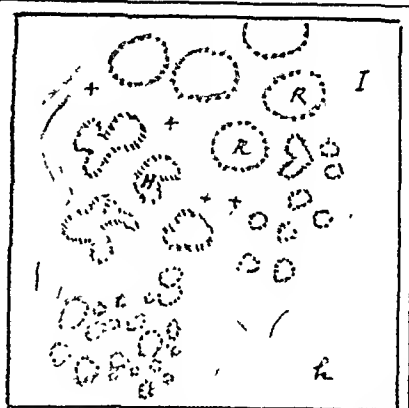


Fig. 3.



Fig. 4.

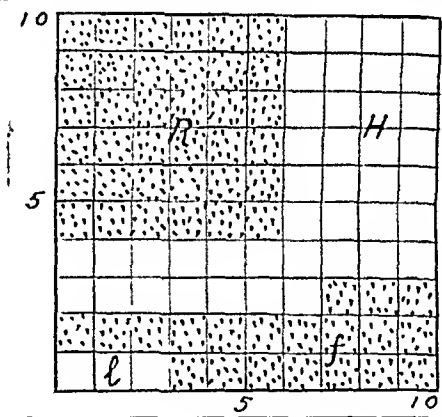


PLATE I.—Figs. 1, 2, 3.—Interpretative sketches. Fig. 1.—Simple colloid, nontoxic goiter. Fig. 2.—Toxic goiter. Fig. 3.—Fetal adenoma.

The letters are the same as used in the text. In Fig. 1, the shaded areas represent either actual acini, or groups of similar acini, according to requirements. The + denotes lymphocytic infiltration. The two lighter areas denote collections of acini whose colloid contents stain less intensely. In Fig. 2, the hyperplastic acini are shown as having more elongated lining cells.

Fig. 4.—Alternative representation, in which the proportions of the different components are given in graphic form: 36 per cent (i.e. 36 squares) of the tissue is made up of acini with normal colloid; 20 per cent is formed of interstitial tissue; 3 per cent (in area of sections) is infiltrated with lymphocytes. The rest of the tissue shows acini with feebly-staining colloid. With this diagram it is easier to put side by side the histologic and clinical pictures of the given case.

ing adrenal cortical cells; and we allow for degenerative changes, especially in the arteries. Each and all of these components may occur focally "F" (as exemplified by the macroscopic adenoma), or diffusely "D" through the sections.

Using the following simple list of varieties, we may therefore exemplify this method of analysis

VARIETY OF GOITER	FORMULA
1 Simple	$D R (\pm F)$
2 Adenomatous	$n F (H < R) + n D (R \pm f)$
3 Combination of 1 and 2	$n F (H < R) + n D (\sqrt{H \pm} R + z f)$
4 Fetal	$n (F/D) H + n' D R (\lambda I + l, h, f) \pm \text{calc}$
5 Cystic	$F (R + f) + D R$
6 Exophthalmic	$D (\sqrt{H}) + y I + l + h + z f$
7 Malignant	$F H + D (R \pm H + f)$

In the case of paired letters, the first small letter of the pair (n, n', \sqrt{x}, y, z) stands for an unspecified quantity, as in algebra. An attempt at exact numerical values could be made by making actual measurements of a projection of the section upon squared paper, counting out how many squares or parts of squares are covered by the different types of acinus, etc., or even cutting the picture out and weighing the different parts. This refinement would be reasonable only if many sections from one case were subjected to it. For practical purposes a rough graph could be drawn up or an interpretative sketch made in which the spatial disposition of the various components is indicated.

The third example given would be translated thus "there are focal collections or adenomata present, whose acini are predominantly in the resting phase, but there is in volume a greater amount of diffuse change. In the latter there are so many (n) acini in the resting phase, so many (y) in the hyperactive phase, and so much (z) fibrosis."

Needless to say, the use of an algebraic form is only suggested for the laboratory files, for possible future statistical application. But it is the basis of the pathologist's actual official report, a copy of which is also filed in the laboratory.

THE BEARING OF HISTOLOGY ON THE CLINICAL MANIFESTATIONS

It is not the goal of the pathologist simply to record with an approach to mathematical exactitude the composition of his specimen. He seeks to ascertain the meaning of the pictures he presents. It is true that the specification of the picture itself, or the determination of its exact meaning may not alter the surgeon's or physician's practice on the given case in hand. Yet it is not a mere academic pastime to seek the meaning of the histologic features. One wishes to understand the disease, so that in future it is possible to realize just exactly what has gone wrong to produce that particular thyroid enlargement in that particular patient. In one kind of case the function of the gland is normal. It is the rate of change which is accelerated, or slowed down excessively. In another case there is actual dysfunction and this may be interpreted in various ways. For example, on a biochemical basis, it is visualized in terms of iodine metabolism. On a colloidal chemical basis, the iodine per se is not taken as the cause of the symptoms or lack of symptoms, or of the staining effects in the tissue section. It sees the iodine as merely holding the thyroid globulin in place, since lack of iodine caused the protein constituents of the secretion to pass from gel state to sol, this change being what is visible in the stained sections, and accounts for toxic symptoms when the extent of colloid change reaches a certain threshold. A seemingly trivial loss of iodine may thus set the full clinical picture of thyrotoxicosis going in a moment.

The nuances of the histologic picture can now fall into their place, and the apparent discrepancies between clinical symptoms and pathologic report are bridged over. For instance, patches of "R" in the section mean intermittent or periodic iodine deficiencies, the acini having been exhausted at some time, here and there. Diffuse "R" change, means that the iodine deficiency was prolonged. Large acini mean that iodine-supply has been restored, whether by administration of lugol, or spontaneously. Hyperplastic foci in the section mean lack of supply of iodine, which is being met by increased secretion on the part of the thyroid.

Islets have a similar significance. Patches of "I" infiltration mean the presence of a secretion perverted in quality. Fibrosis denotes scarring after previous attacks of hyperactivity. Calcification denotes precipitation of colloid into inorganic irreversible material.

Working backwards, it would be possible for the pathologist to point out in the section, or in his finished diagram, the relation of the particular symptoms of the case as being the result of such and such biochemical processes or regressions, remembering always that the various distinctive symptoms do not arise until the amount of change reaches a certain threshold and unless there is an imbalance in the past of the associated incursions.

TABLE I

FREQUENCY	FOCAL				DIFFUSE		
	ADENOMATOUS		CYSTIC		DR.		DI.
	TOXIC	NOTTOXIC	TOXIC	NOTTOXIC	TOXIC	NOTTOXIC	TOXIC
Commonest age	23%	15%	4%	2%	35%	11%	9%
History over 2 yr.							
	30-45	30-45	early and late	30-45	14-20	30-45	14-20
	15%	6%	4%	15%	33%	8%	None
Symptoms	Pressure	+	-	+	+	+	-
	Cardiac	+	+	+	+	-	+
	Nervous	+	+	-	+	+	+
	Exophthalmos	-	-	-	+	-	+
Postoperative trouble	66%	8%	1%	1%	13%	2%	4%

Table I may serve to indicate how an analysis of histologic results harmonizes with an analysis of the clinical findings in a small series of cases of goiter (studied at St. Paul's Hospital, Saskatoon, Sask.).

SUMMARY

The subject of goiter is taken as a convenient example for showing how complex tissue changes can be represented in the records of a surgical pathologic laboratory. A similar method can be utilized for other types of material (appendicitis, urologic, gynaecologic, etc.).

In the case of goiter, it may be thought that the achievement of exactitude in histologic analysis should be the point at which the pathologist should fade out of the picture, leaving the field to the surgeon and physician. However, this is really the point at which the true function of the pathologist should begin. Even were that point to be the limit of achievement allowed, it would still be true that by this method the pathologist is making for a truer insight into the real nature of the disease in some particular patient, to whose ultimate advantage the work is directed.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

RENAL FUNCTION, Tests for, Don, C S D Brit M J p 54, July 10, 1937

Renal function tests have been carried out in sixty four cases in which it was thought that kidney disease might be present. The tests used were the blood urea and the urea clearance test, the maximum specific gravity test, and the phenolphthalein test. In the first group of thirty three cases, some loss of renal function was demonstrated, and the patients comprising this group were on the whole more incapacitated and death was more common than among the second group of thirty one cases, in which no loss of renal function was observed. Many of the patients were followed up after their discharge from the hospital. On clinical grounds alone some of those included in the first group because of loss of renal function might have been included in the second group without causing the least surprise. The reverse is equally true.

With pronounced renal failure, as indicated by a high blood urea the urea clearance and the phenolphthalein tests showed marked depression of renal function, but this was not true of the maximum specific gravity. On two occasions it was normal, or nearly so, when the blood urea was over 100 mg. In both cases there was edema, and the patient was passing very little urine. The ability to secrete urine was at fault, but the ability to concentrate was normal. In some cases where renal function was apparently not nearly as severely impaired, this test seemed to be very delicate, the same may be said of the urea clearance test and the phenolphthalein test, each in some cases appearing to be the most sensitive. A surprising number of patients showed a lowered phenolphthalein excretion, but this may possibly be due to the fact that many had a high blood pressure. The lack of parallelism between the tests, especially in the lesser grades of renal damage, was most disappointing. None of them appear to be capable of enabling the clinician to give a very much more accurate prognosis than is possible from a knowledge of the blood urea alone. However, if any of the tests show marked loss of renal function in the absence of a raised blood urea, it would probably be best to give a guarded prognosis. A reexamination of the patient in three months might help. Clinical examination will often furnish very valuable information, and must still be regarded as of the foremost importance.

To conclude, the failure of the tests to agree closely with one another suggests either that they are not completely reliable or that different renal functions are being measured.

SCARLET FEVER Immunization Against, Peterman, M G Am J Dis Child 54 90, 1937

The author summarizes his brief against the present method as follows:

The bacteriologists and most clinicians do not accept a specific strain of the hemolytic streptococcus as the etiologic agent of scarlet fever.

The bacteriologists insist that it is not possible to produce an active immunity against any streptococcus.

The specificity of the Dick test has not been proved.

Active immunization with the Dick toxin or vaccine prevents the development of a rash for two or more years. If the inoculated person becomes infected with scarlet fever, his physician is robbed of his most valuable diagnostic sign.

The Committee on Clinical Investigation and Scientific Research of the American Academy of Pediatrics reported on June 11, 1934: "The whole matter of scarlet fever immunization, both by the toxin and by the toxoid methods, is being investigated . . . by the U. S. Public Health Service and the Massachusetts State Board of Health. The results of these investigations must be awaited before it is possible to evaluate their usefulness." In April, 1935, the Special Committee on Prophylactic Procedures Against Communicable Diseases of the American Academy of Pediatrics reported: "Active immunization (against scarlet fever) . . . is not recommended as a general public health procedure, since reactions both local and general are frequent . . . and for the reason that the degree and duration of immunity have not been definitely established."

PREGNANCY, The Chemical Diagnosis of Early, Patterson, J. Brit. M. J., p. 522, September 11, 1937.

A biochemical test for the diagnosis of early pregnancy is described which is based upon bacterial splitting of estriol glycuronide and the subsequent development of the estriol color reaction with phenolsulphonic acid.

Urines from sixty-five cases in which pregnancy diagnosis was required have been examined by the test. In all except one case the result was in agreement with that of the Friedman reaction. The advantages and disadvantages of the test as compared with the biological tests are briefly discussed. The technique follows:

Urine Sample: The requirement of the test is a sample of at least 50 c.c. of early morning concentrated urine, collected into a vessel chemically clean in the sense of being entirely free from all substances which might be inhibitory to bacterial action. It is also advisable that the patient should not have had any drugs for a few days before the collection, for these often increase the pigmentation of the urine, and may also adversely affect the preliminary bacterial hydrolysis.

Preliminary Treatment: The urine is tested for high acidity by treating a few drops with methyl red, and if acid to this indicator, it is adjusted with addition of alkali until it no longer shows a pink color. A 50 c.c. sample is then heavily inoculated with *B. coli* and incubated overnight. To the urine after incubation, while it is still warm, is added approximately 0.5 gram of sodium bisulphite. The specimen is shaken until the solid has completely dissolved and then allowed to stand for fifteen minutes.

Extraction: The partially decolorized urine is transferred to a separatory funnel of about 150 c.c. capacity and extracted with two lots of 40 c.c. of ether. Occasionally some emulsification occurs, but this may be rapidly resolved by centrifugation. The combined ether extracts are washed with a little water, and then well shaken with a 30 c.c. portion of 10 per cent sodium carbonate. The alkali layer having been discarded, this washing process is repeated until the carbonate layer is completely colorless. The ethereal solution is then again washed with distilled water, and when the water layer has been drawn off, the ether layer is further extracted with two lots of 40 c.c. N/10 sodium hydroxide. After separation and rejection of the upper ethereal solution, the combined alkali portions containing the estriol are treated with 25 per cent sulphuric acid drop by drop until acid to Congo red paper. This acidified aqueous solution is then extracted with two portions of 40 c.c. of pure "analytical" ether, after which the ether layers are combined and washed with a little water. The ether extract is given another washing with 10 per cent sodium carbonate, and after the rejection of the alkali, the remaining ether is freed from all trace of the alkaline carbonate by two more washings with distilled water. The final clear, colorless ether solution is next transferred in two portions to a 50 c.c. transparent silica flask, and the solvent completely evaporated by immersing the flask in a large beaker of water previously heated to 70° C.; the last traces of moisture are then removed in vacuo by direct application of suction to the flask. The dry residue is now ready for the development of the color reaction, using the phenolsulphonic reagent.

Reagent The reagent used is that recommended by Cohen and Marrian, and consists of 36 parts of pure phenol with 56 parts of pure concentrated sulphuric acid. Small quantities only are prepared at a time, 9 cc of phenol, liquefied by heating, to which are added 14 cc of sulphuric acid with sufficient external cooling to the container to prevent undue rise of temperature, makes a convenient amount. It can then be stored in a 25 cc all glass buret, fitted with a ground glass stopper so as to exclude moisture from the reagent. Considerable care must be taken with the reagent, which is very hygroscopic and becomes inactive when it takes up water. In measuring out, therefore, it is important first to discard a volume equal to that portion in the tip of the buret below the stopcock, so that only completely protected reagent is used for the reaction. Moreover, it is advisable only to use a reagent which is less than one week old.

Color Reaction To the flask containing the dry residue, 1 cc of reagent is added from the buret, and the flask then immersed in a large water bath, previously heated to about 70° C. By frequently rotating the flask, the whole of the residue distributed around the sides is brought into the reagent, and the temperature of the bath is rapidly raised to boiling point and kept there for a period of exactly ten minutes. During the boiling period, it is again advisable from time to time to rotate the reagent around the sides of the flask. The reagent at this stage has assumed a yellowish color and it is now cooled by holding the flask under a stream of tap water. While this cooling process is going on, 1 cc of 5 per cent sulphuric acid is slowly added, the contents of the flask being kept moving in order to bring the somewhat syrupy reagent into a homogeneous solution with the dilute acid. The product, which is still yellowish, is then reheated in the boiling water bath for a period of two and a half minutes. A positive reaction is obtained when the original color gradually changes over to pink or red, a negative reaction, when this change is entirely absent.

MONONUCLEOSIS, Infectious Diagnostic Value of Supravital Staining in, Gall, E. A. Am J M Sc 194 546, 1937

There is fairly generally admitted difficulty in definitely identifying the specific cell of infectious mononucleosis.

Despite the evident value of the determination of the presence of heterophile agglutinins, recognition of characteristic morphologic features of the typical cell of the disease would obviate its use to a great extent. Such features are evident in supra-vital stained preparations.

By this means the cell is definitely shown to be an atypical but relatively mature lymphocyte, readily distinguished from other mononuclear cells by several characteristics.

Refractile granules present in one third of the lymphocytes in other diseases and in the normal state are never present in higher than 15 per cent of the lymphocytes in infectious mononucleosis.

ANTHRACOSILICOSIS, Carcinoma of Bronchus in Association with, Charr, R. Am J M Sc 194 535, 1937

Clinical and pathologic observations on 4 cases of primary carcinoma of the bronchus associated with anthracosilicosis are briefly described.

Dyspnea, hemoptysis, cough, expectoration, and pain in the chest were the outstanding symptoms. Sputum negative for tubercle bacilli (with hemoptysis a frequent symptom) excited a suspicion of primary malignant growth of the bronchus.

Röntgen ray diagnosis of diffuse bronchiogenic carcinomatosis is difficult when anthracosilicosis coexists.

Extensive destruction of the bronchial cartilages, resulting in collapse of the involved bronchi, might have been responsible for severity of dyspnea.

Capability of the peribronchial and perivascular lymphatics to dilate in spite of fibrosis surrounding them was noted.

Spread of tumor cells through the alveolar pores and interlobar adhesions was observed.

Absence of toxic symptoms of pulmonary suppuration was attributed to impaired lymphatic absorption and lessened blood flow through the atelectatic lungs.

A suggestion is made that in these cases anthracosilicosis might have had an indirect influence on development of carcinoma of the bronchus.

CHANCROIDAL TEST, Diagnostic Value of the Intradermal, Greenblatt, R. S., and Sander-son, E. S. Arch. Dermat. & Syph. 36: 485, 1937.

A total of 721 intradermal chancroidal tests were performed upon 191 patients, and conclusions are drawn as to the diagnostic value of the test.

The superiority of the chancroidal test with bacillary antigen, as advocated over the test with antigen prepared from the pus from buboes as described by Cole and Levin, is evaluated.

A warning note is sounded to investigators who report cases of lymphogranuloma venereum on the basis of a positive Frei test without performing a concurrent chancroidal test. Twenty-four patients in a group of 80 patients exhibiting a positive reaction to the chancroidal test also had a positive reaction to the Frei test; this emphasized the need for caution in the interpretation of the results of these tests.

A positive chancroidal test is only presumptive evidence and does not necessarily mean that the lesion under consideration is chancroidal. A similar conclusion should be borne in mind in the interpretation of a positive Wassermann as well as of a positive Frei test in every case of genital lesion, unless other confirmatory laboratory evidences are present.

Finally, a negative intradermal test with bacillary antigen is diagnostic so far as it rules out infection by the bacillus of Ducrey. A positive intradermal chancroidal test commits one to the diagnosis of chancroidal infection only when the Frei test, the Wassermann test, and the microscopic examinations for malignant disease and granuloma inguinale give negative results.

TRICHINOSIS, Studies On, Hall, M. C., and Collins, B. J. Public Health Reports, 1937.

Hall and Collins report upon a study of trichinosis conducted under the auspices of the National Institute of Health, United States Public Health Service.

A study of 300 diaphragms from cadavers, coming from 10 hospitals in Washington, D. C., and 1 hospital at Baltimore, Md., shows 41 diaphragms infested with trichinae, an incidence of 13.67 per cent.

The samples include cases from 5 Federal hospitals to which patients are sent from all over the United States, and from 6 Washington hospitals with cases originating widely over the United States. The cases run the range of childhood to old age, military and civil life, association with land and sea, sane individuals and mentally deranged hospitalized cases, black and white, male and female, and high and low economic-social status.

All diaphragms were examined by both the direct microscopic method and by the digestion-Baermann method, since both methods have special value for certain types of infestation and both have certain limitations, the two methods being supplementary in these respects.

On the basis of 1,778 cases reported up to the present time, the writers conclude that an indicated incidence of approximately 12.5 per cent, an unweighted average, is a conservative figure, probably definitely too low. If this figure is indicative of incidence throughout this country, there are probably several million persons in the United States who are infested with trichinae, among whom are possibly several hundred thousand who have had clinical trichinosis never diagnosed as such, and there are possibly several thousand deaths annually from this cause.

The following point is emphasized: The United States apparently has the greatest problem of trichinosis of any country in the world, a problem involving, in one way or another and in some degree, several million persons. The incidence in man is greater than the incidence in garbage-fed hogs.

A consideration of the age incidence by decades indicates that, at least up to some unascertained point, there is an increased incidence with increasing age, due apparently to the

fact that an increase in the time factor increases the opportunities for infection. At some unascertained point there may be a mortality factor in the form of deaths occurring at an age earlier than would have been the case had it not been for pathologic conditions persisting after recovery from trichinosis, thereby removing from the older age groups some of the positives that might otherwise have appeared there. Positive findings in recent literature indicate the advisability of examining suitable muscle tissue from very young infants and those prematurely born, for the possibility of detecting prenatal infection with trichinae. The post mortem study of suitable muscles from persons dying after prolonged confinement in hospitals, jails, and penitentiaries, under modern sanitary conditions precluding the eating of raw or undercooked pork, is suggested as a basis for obtaining more precise information as to the time larval trichinae survive alive in human beings, and the time required for calcification of the cysts and for the death of the trichinae. It is recommended that the microscopic examination of 1 gram of diaphragm muscle, as a press preparation and not by sectioning, be made a routine procedure in the postmortem examinations by pathologists.

Since examinations of 1,778 cadavers at 24 hospitals in 11 places in the United States indicate an incidence of at least 12.5 per cent of trichinosis, with not 1 case out of 222 positive cases having been diagnosed as trichinosis at any time, it is evident that knowledge of the polymorphic picture of clinical trichinosis is inadequate and that we need more information in regard to diagnosis.

To clarify the picture of trichinosis, especially of atypical clinical cases caused by infestations of intermediate extent, as opposed to very heavy or very light infestations, calls for much research and cooperation by pathologists, clinicians and parasitologists. Quantitative studies are especially necessary.

Gorbage fed swine have trichinae between three and five times as frequently as do grain fed swine, and hence are especially important as sources of human trichinosis.

Trichinosis in swine is apparently traceable to the eating of uncooked pork scraps in garbage, table scraps, swill, and similar things, much more often than it is traceable to the eating of ruts by swine.

The garbage feeding industry, as ordinarily carried on, is dangerous to the health of man and livestock, is esthetically objectionable, and is often economically unsound.

Suggestions are made for the elimination of the dangers and nuisances associated with the garbage feeding industry and with the feeding of table scraps and similar things on the farm. Cooperation between scientists, practicing physicians, engineers packers and the swine industry is recommended as the best attack on the problem.

The authors are emphatic in recommending the examination of excised tissue as a press preparation rather than by section, regarding the former method as not only more rapid but more accurate. They emphasize, also, the need for development of better methods of early diagnosis, especially in the atypical case.

PNEUMONIA DUE TO PNEUMOCOCCUS TYPE VII Bullowa, J. G. M., and Greenbaum E. Arch. Int. Med. 60: 180, 1937

It appears as the result of these studies that among the patients who received serum the mortality was significantly reduced and the duration of illness was shortened, even when serum treatment was begun on the fifth, sixth, or seventh day of illness.

The patients to whom serum was administered before the fifth day of illness had no complications. For the group of patients who received serum on the fifth day or later and for the group who were not given serum, the incidence of complications was approximately the same, but the mortality among those with complications who were treated on the fifth day or later was lower than the mortality among the corresponding group of patients who did not receive serum treatment.

STAPHYLOCOCCIC IMMUNITY, Rigdon, R. H. Arch. Path. 24: 233, 1937

In this review of staphylococcal immunity, it has been found that vaccines have very little therapeutic effect on staphylococcal infections either experimentally or clinically. It is suggested, however, that they may stimulate the formation of antibodies in man.

Toxoid appears to be a very efficient antigen in the treatment of certain staphylococcal infections of low virulence in the skin and may be of some value in certain cases of chronic infections, such as osteomyelitis. Toxoid produces an increase in the circulating antihemolysins, and this is frequently accompanied by clinical improvement.

The experimental and clinical results suggest that staphylococcus antitoxin has a definite therapeutic value in treatment in certain cases of acute staphylococcal infections which are accompanied by toxemia. This antitoxin may act in the following ways: The toxin produced by the staphylococcus in vivo is neutralized by this antitoxin; a component of the immune serum may act on the staphylococci and increase the ease with which they are phagocytosed by certain organs, especially the liver and spleen. The antitoxin in the circulation may also inhibit the action of leucocidin and thus allow the polymorphonuclear leucocytes to phagocytose the bacteria in either the blood or the tissue.

From the experimental observations on the mechanism of immunity to staphylococcal infections, it appears that vaccine, toxin, or toxoid may also cause proliferation of the lymphoid and histiocytic elements, especially in the liver and spleen. This cellular change enhances the ability of these tissues to phagocytose the staphylococci.

MYXEDEMA, Anemia of: Classification and Treatment, Sharpe, J. C., J. A. M. A. 194: 382, 1937.

The exact etiology of the anemia of myxedema is unknown, but it is probably the result of decreased blood formation, due to the hypometabolic effect on the bone marrow.

In addition to the usual physical signs of myxedema, the anemic patient may present such clinical features as a lemon yellow pallor, glossitis, paresthesias, and an achlorhydria. An erroneous diagnosis of pernicious anemia may be made.

In 3 cases, the mean corpuscular volume and the hemoglobin content of the red blood cells showed that the anemia was normocytic and normochromic in character. In the fourth case an achlorhydria, hypochromia, and microcytosis may have been due to an associated iron-deficiency anemia.

Though thyroid extract causes a prompt increase in the basal metabolic rate, a transient decrease in the blood count may first be noted, followed by a slow and sustained rise in both hemoglobin and erythrocytes.

In the presence of hypochromia, iron may accelerate the regeneration of blood cells, but liver extract appears to be of no value in this type of anemia.

HYPERTENSION, Arterial, Site and Significance of High Chloride Content of Blood, Apperly, F. L., and Cary, M. K. Am. J. M. Sc. 194: 352, 1937.

In a study to determine the site of increased blood chloride in patients with arterial hypertension, and if possible its significance, it was found:

1. The increased blood chloride is wholly confined to the red cells.
2. This increased cell chloride is not the result of acidemia, since pH and erythrocytic volume-index showed no significant deviations from the normal.

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Though thyroid extract causes a prompt increase in the basal metabolic rate, a transient decrease in the blood count may first be noted, followed by a slow and sustained rise in both hemoglobin and erythrocytes.

In the presence of hypochromia, iron may accelerate the regeneration of blood cells, but liver extract appears to be of no value in this type of anemia.

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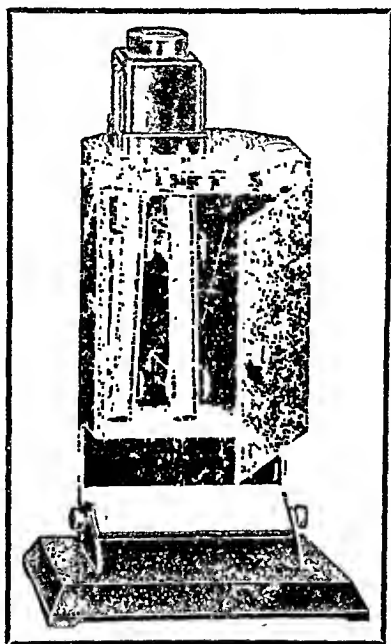
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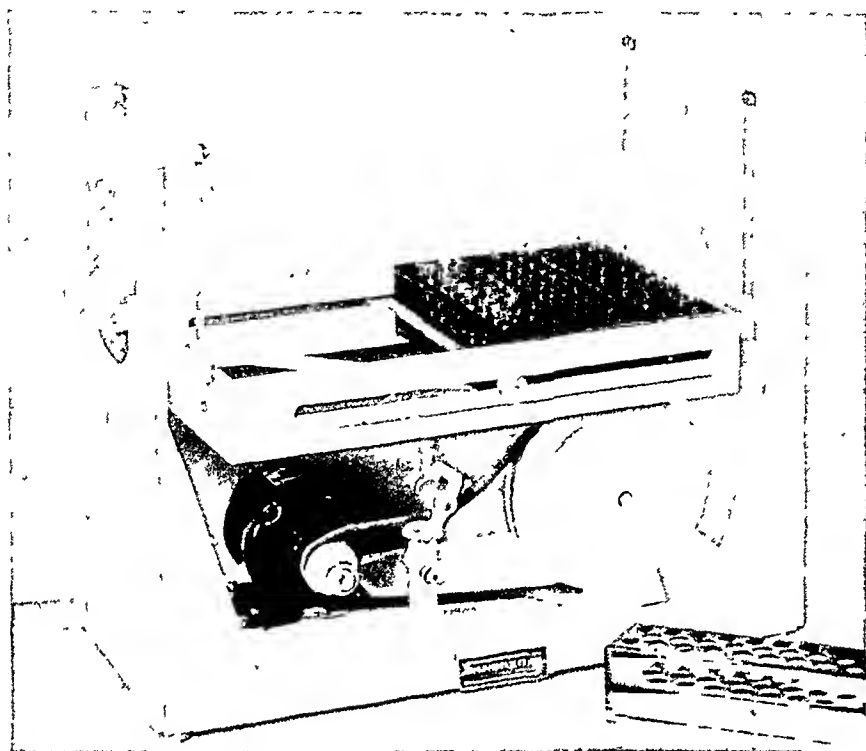
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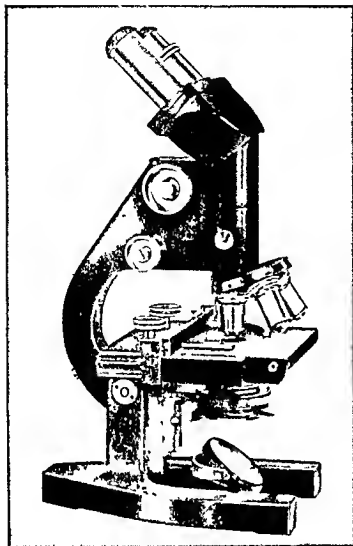
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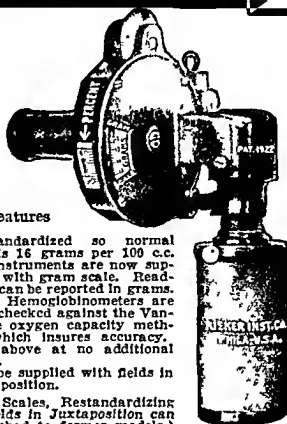
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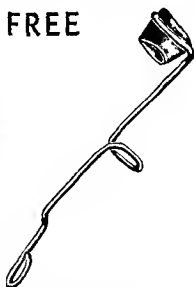
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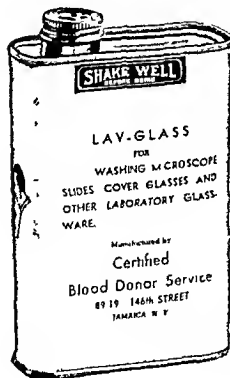
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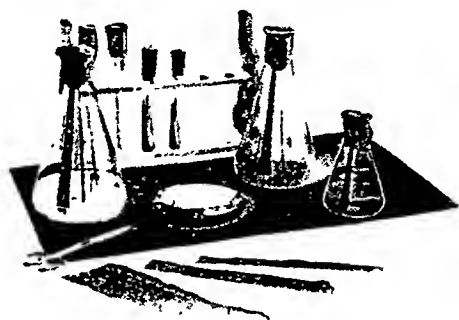
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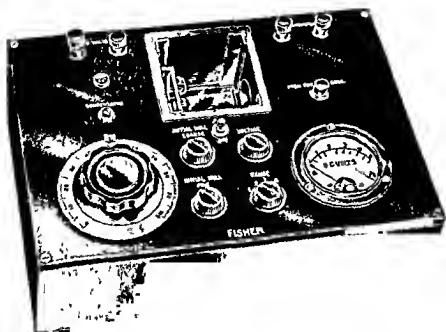
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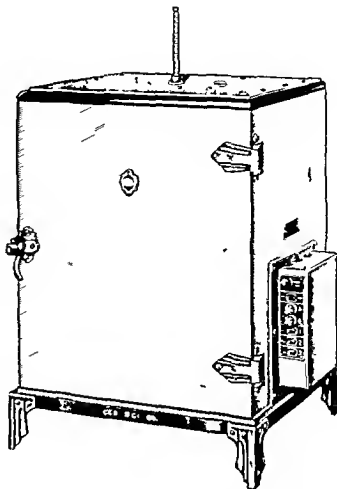
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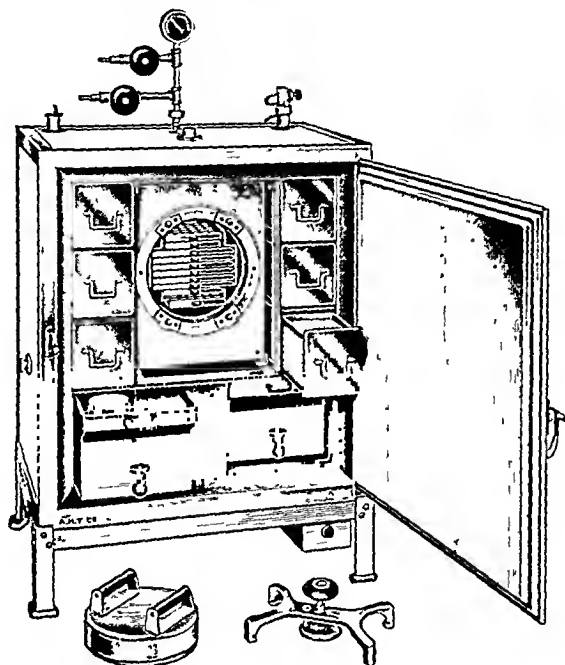
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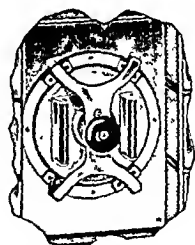
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The Journal of Laboratory and Clinical Medicine

VOL 23

FEBRUARY 1938

NO 5

CLINICAL AND EXPERIMENTAL

DOES REMOVAL OF A NORMAL GALLBLADDER AFFECT THE METABOLISM OF LIPIDS?*

A J ATKINSON, M S M D, AND A C INY, PH D, M D CHICAGO, ILL

TWO general functions are generally assigned to the gallbladder, namely, the regulation of pressure of the biliary passages, and storage of bile for digestive purposes. Certain experimental observations have been reported by Sweet¹ which indicate that the gallbladder may be related to lipid metabolism. His data are sufficiently striking to warrant a repetition and extension of his experiment.

Sweet fed a normal dog a fat meal and determined the cholesterol content of the blood at three hour intervals for twelve hours. Having determined the normal cholesterol curves he removed the gallbladder. He found that after cholecystectomy the blood cholesterol following a fat meal rose to almost double the normal values. About forty days after operation the blood cholesterol response returned to normal, and was less than normal at seventy four days. In one observation three hundred days after operation, the cholesterol curve approached normal. He also noted a change in the contour of the cholesterol curve following fat ingestion. Normally the peak of the rise occurred at the sixth hour, whereas after cholecystectomy the peak was reached at the ninth hour. The increase in blood cholesterol was reported to be due chiefly to a rise in cholesterol esters.

Fowweather and Collinson² found the blood cholesterol values in man normal or subnormal several months after cholecystectomy. This is not necessarily contrary to Sweet's findings in dogs.

*From the Departments of Medicine, Physiology, and Pharmacology, Northwestern University Medical School.

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We thought it possible that a more continuous flow of bile into the intestine after removal of the gallbladder might modify the absorption rate of fat. However, not only the rate of absorption of lipids into the blood might be a factor in interpreting Sweet's results, but other factors such as tissue injury might modify the alimentary lipemia curve. With these possibilities in mind, the experiment of Sweet has been repeated and extended.

METHODS

Normal absorption curves in dogs for fat similar to the dextrose tolerance test were determined. The dogs were fasted for three days because Rony and Ching³ obtained more uniform alimentary blood fat curves after three days of

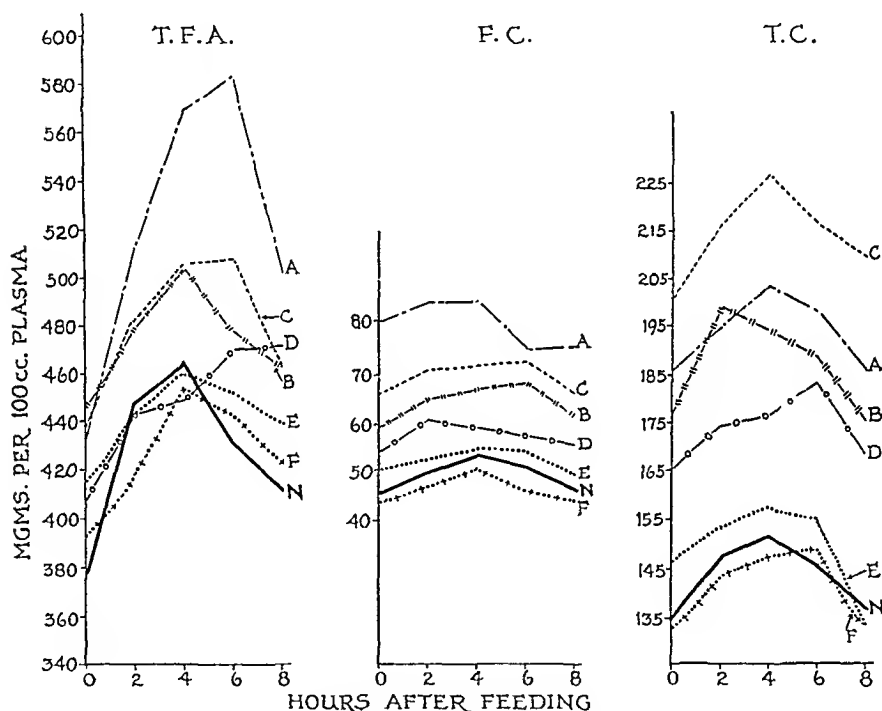


Fig. 1.—A composite curve of nine dogs showing the effect of cholecystectomy on blood lipids. T.F.A., total fatty acids; F.C., free cholesterol; T.C., total cholesterol. N, control curve; A, six days after operation; B, twenty-four days; C, forty-two days; D, sixty-seven days; E, six months; F, one year.

fasting. Then the blood plasma was analyzed for free cholesterol by M. Yasuda's⁴ method, for total cholesterol by R. Okey's⁵ method, and for total fatty acids by W. R. Bloor's⁶ process. The dogs were then given by stomach tube 2 c.e. of olive oil per pound body weight, and four more blood specimens were taken at two-hour periods for analysis. After obtaining several normal blood lipid curves the gallbladder was removed and the experiments were repeated at intervals for several months.

Mange had to be avoided because it was found that three days of fasting in the presence of mange augmented the fasting level of plasma lipids. The same will probably occur if the dog is not given good dietary and hygienic care.

RESULTS

Repetition of Sweet's Experiment—Nine dogs were studied according to the experimental outline just given. Composite curves of the results are shown in Fig 1. These curves are not unlike those obtained by Sweet, and constitute essentially a confirmation of his observations.

It was found that in the dogs operated upon there was an increase in the third or sixth day fasting level of blood lipids. However, the blood lipid curves

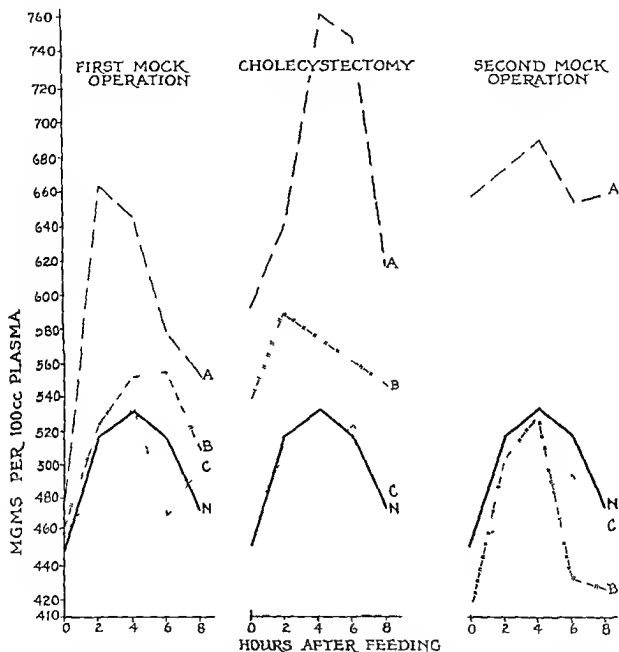


Fig 2—A composite curve of four dogs showing the effect of a mock operation, cholecystectomy and a second mock operation on total fatty acids. *N*, normal control. *A*, three days postoperatively. *B*, twenty days. *C*, thirty two days after mock operation, eighty five days after cholecystectomy and second mock operation.

after fat ingestion were not absolutely uniform either before or after cholecystectomy, and the peak of the rise did not occur at a definite hour. The fasting levels occasionally returned to normal within a month after operation. In one dog the third day fasting level of total fatty acid and total cholesterol remained high for one year after cholecystectomy. This dog for some unknown reason remained in poor condition after the operation. We consider the "A," "B," and "C" curves to be significant elevations of the lipid values.

A Control Group of Experiments.—"Mock operation": It was then thought advisable to determine whether the results obtained were specifically due to removal of the gall bladder, or whether they could be produced by stripping out a piece of the hepatic capsule and manipulating, without removing, the gall bladder. Such a control was considered to be important because of the known relation of liver injury to blood plasma cholesterol values.

The results on four dogs in general were similar to those obtained by cholecystectomy. The "mock operation" was followed by a rise in the level of blood lipids after three days of fasting. The response to fat feeding also was modified as if the gallbladder had been removed.

Of course, the manipulation of the gall bladder may have injured it. So, experiments were performed on four dogs on which a "mock operation" was

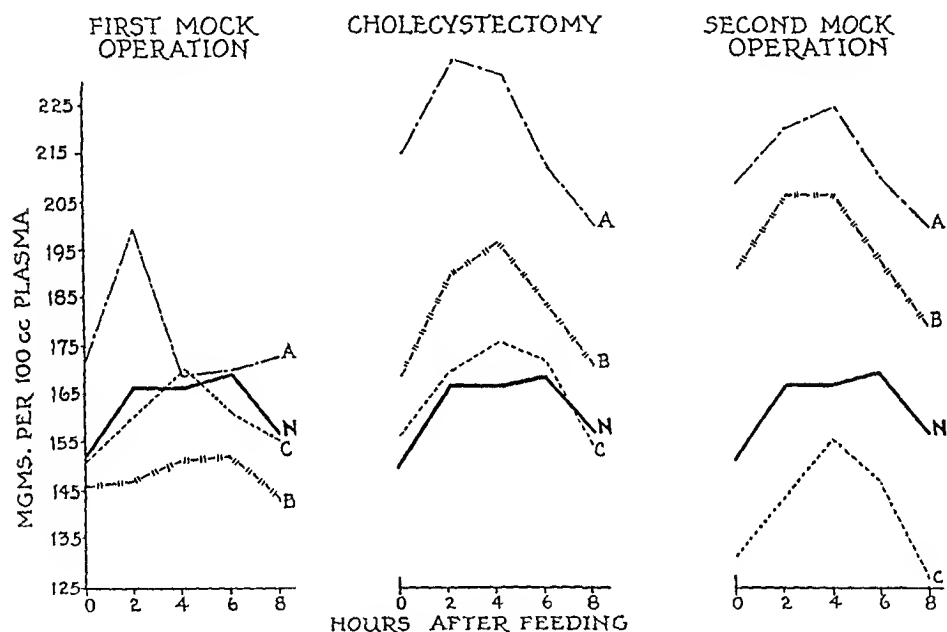


Fig. 3.—A composite curve of four dogs showing the effect of a "mock operation," cholecystectomy, and a second mock operation on total plasma cholesterol. N, normal control; A, three days postoperatively; B, twenty days; C, thirty-two days after mock operation, eighty-five days after cholecystectomy and second mock operation.

performed without manipulating the gallbladder. However, after the plasma fat changes had returned to normal, the gallbladder was removed, and then after the plasma fat changes had returned to normal another "mock operation" was performed.

The essential results are shown in the composite curves in Figs. 2, 3, and 4. More frequent tests were performed than those indicated in the figures, but it is unnecessary to take space by the presentation of all the data. The curves, however, are an accurate representation of the average trends in the various animals. It is to be noted that the mock operations influenced the results less than the cholecystectomy. Also, the effect of cholecystectomy tended to be longer in duration.

Another dog was fed a quantitative diet every day. The twenty four hour postcibal blood fat levels were determined successively before and after ether anesthesia, trauma to the liver but not the gallbladder, and subsequent cholecystectomy. At times this dog showed some increase in the twenty four hour postcibal blood lipid values, but on the average the levels were but slightly higher than is the normal variation for the same dog (Table I). This dog showed a poor response to cholecystectomy.

TABLE I

THE RESULTS OF CHOLECYSTECTOMY AND OTHER PROCEDURES ON BLOOD FAT LEVELS TWENTY FOUR HOURS POSTCIBUM

DATE	TOTAL FATTY ACID MG / 100 CC	FREE CHO LESTEPOL MG / 100 CC	TOTAL CHO LESTEPOL MG / 100 CC	AVERAGE TOTAL FATTY ACID	AVERAGE FREE CHOLE TEPOL	AVERAGE TOTAL CHOLE TEPOL	AVERAGE CHOLE TEPOL ESTER
4/20/35	363	4	110				
4/22/35	777	32.4	107				
4/24/35	508	53	163				
4/26/35	393	41.8	115				
4/29/35	734	40.8	118				
5/ 1/35	408	40.8	113	399.7	40.9	120.8	80
5/ 3/35	393	43.9	118				
5/ 5/35				Dog fasted 48 hours			
5/ 6/35	362	41.8	131				
5/14/35				Deep ether anesthesia for one hour			
5/15/35	470	44.9	152				
5/17/35	524	31.4	115				
5/20/35	39	37.4	94				
5/22/35	300	34.5	105				
5/24/35	339	32.4	110				
5/27/35	402	50.7	84	421.8	77.9	112	74
5/29/35	424	74.5	115				
5/31/35	454	42.8	107				
6/ 3/35	431	48.0	128				
6/ 6/35				Laparotomy, manipulated gallbladder (ether anesthesia)			
6/ 7/35	501	46.0	121				
6/10/35	487	51.2	131				
6/12/35	501	44.9	115				
6/14/35	723	4.9	173	445	46.8	146	99
6/17/35	416	48.1	191				
6/18/35				Removed gallbladder (ether anesthesia)			
6/19/35	447	52.3	173				
6/21/35	454	70.0	170				
6/24/35	534	69.0	173				
6/26/35	431	50.2	131				
6/28/35	447	42.8	155				
7/ 1/35	385	39.7	89	440.03	50.7	131	80
7/ 3/35	408	40.8	113				
7/ 5/35	470	47.0	92				
7/ 8/ 5	416	50.2	113				
7/10/35	470	41.8	94				
7/12/35	362	54.2	141				

Thirty pound male dog. Quantitative diet 300 cc milk, 10 cc cod liver oil, 12 gm yeast 200 gm bone, 200 gm Purd (Swift & Co analysis. Protein 10.5 per cent, carbohydrate [N F E] 10 per cent, fat 2.50 per cent, crude fiber [max.] 50 per cent)

Two other dogs were fed the quantitative diet, but were fasted for the days before determining blood fat levels. When this had been repeated several times, a laparotomy was performed, and the fasting and alimentary blood lipid levels were elevated.

When the levels again approached the normal, a cholecystectomy was performed. Again the blood lipid levels were elevated. When the levels had returned to normal after the second operation, another laparotomy was performed. The ensuing fasting blood lipid levels were elevated. Fig. 5 shows the composite curves of two dogs before operation, after laparotomy, after cholecystectomy, and after a subsequent laparotomy. It should be remembered that these

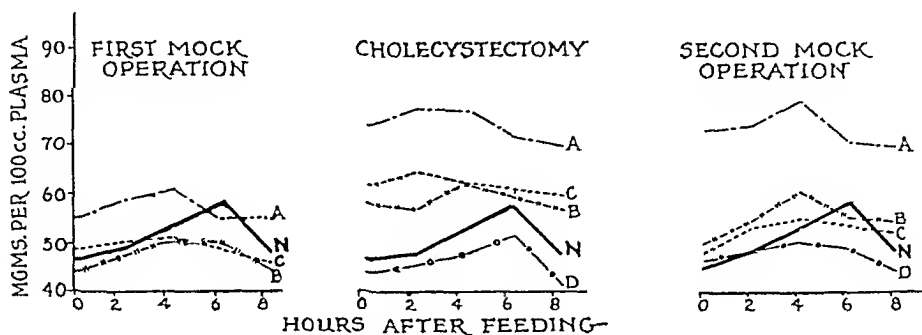


Fig. 4.—A composite curve of four dogs showing the effect of mock operation, cholecystectomy and a second mock operation on free plasma cholesterol. The lettering of the curves means the same as indicated for Figs. 2 and 3.

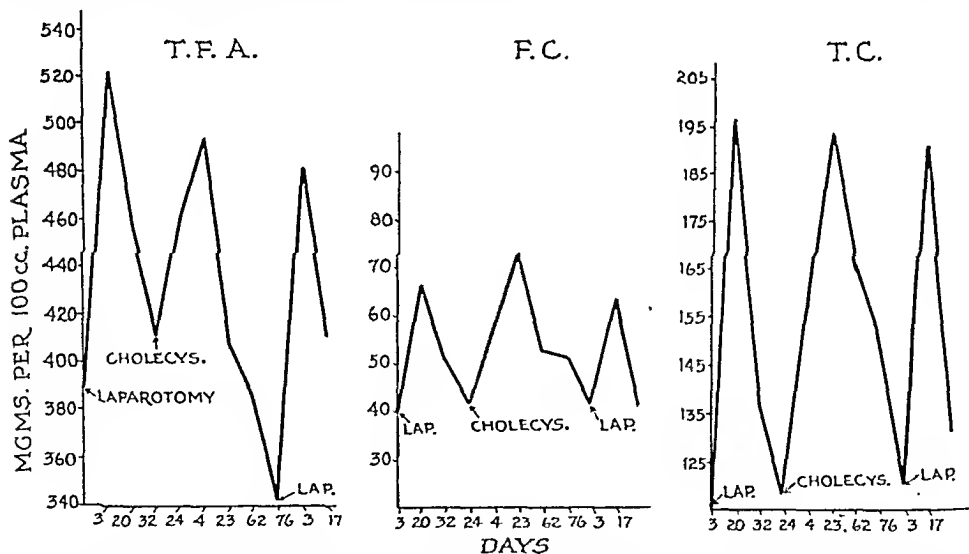


Fig. 5.—Showing the changes in total fatty acids (T.F.A.), free cholesterol (F.C.) and total cholesterol (T.C.) after a laparotomy with liver trauma, cholecystectomy, and a second mock operation.

dogs were fasted for three days before each study of the blood lipid levels. It is to be noted that in this group the laparotomy was as effective in causing changes as the cholecystectomy.

Although the twenty-four-hour postcibal blood fat levels were unchanged after ether anesthesia, the effect of a three-day fast after anesthesia was unknown. Four dogs were fed a quantitative diet and then the blood lipid levels were determined after fasting for three days. In two of the dogs the alimentary lipemia response to olive oil was also studied. After establishing the

normal averages for the blood fats under these conditions each dog was anesthetized with nembutal, ether and chloroform in turn. Nembutal anesthesia did not change the third day fasting lipid levels nor modify the lipemia curve in response to olive oil ingestion. Ether and chloroform anesthetics both raised the third day fasting levels and the alimentary lipemia curves. Chloroform had a more marked and prolonged effect than ether, although the effects were not evident one or two weeks later.

DISCUSSION

It is not surprising that slight injury to the liver changes the blood lipid response to starvation or to ingested fat. In some of the dogs, the effect of cholecystectomy was of longer duration than that of liver trauma. Cholecystectomy is accompanied by slight liver damage, which occurs late, since Bergh, Sandblom, and Ivy⁶ have shown that following removal of a normal gallbladder in a dog small areas of necrosis, undetectable by means of clinical tests, are found in the liver.

This work was undertaken with the hope that some general function of the gallbladder as related to lipid metabolism might be discovered. The results do not indicate that the gallbladder possesses such a general function. Any change that occurred following cholecystectomy can be attributed clearly to the resulting liver injury.

Since cholesterol is eliminated by the intestinal mucosa to a very significant extent, it would be of interest to determine the effect of cholecystectomy on the elimination of cholesterol and fat in the feces. Until this is done one cannot say that the reservoir function of the gallbladder plays no rôle in the metabolism of lipids.

CONCLUSION

The rise in fasting plasma lipid levels obtained after cholecystectomy is not specifically due to removal of the gallbladder, it may also be obtained after liver damage, tissue injury, and for a short time after ether or chloroform anesthesia. By studying the blood lipids (total fatty acids, free and total cholesterol) before and after cholecystectomy both under the condition of fasting and during the digestion and absorption of fat, it could not be demonstrated that the gallbladder possessed a function of regulating the metabolism of lipids.

We would like to express appreciation to Mrs. Vivian Bass for technical assistance in this work.

Part of the expense of this investigation was defrayed by contribution from the E. L. Davies Fund.

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THE PRODUCTION OF PHOSPHATE RICKETS IN RATS IN THE PRESENCE OF VITAMIN D*

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A CRITICAL review of the literature gives one the impression that phosphate rickets can not be produced in rats in the presence of adequate vitamin D. A diet of much higher calcium to phosphorus ratio than has previously been used was planned. The following diet is very low in phosphorus and protein, adequate in calcium, and fulfills the remainder of the nutritional requirements of rats.

TABLE I

FOOD	PER CENT IN RATION	PHOSPHORUS IN GM. IN 100 GM. OF RATION	CALCIUM IN GM. IN 100 GM. OF RATION
Corn	76	0.144	0.014
Oats	20	0.079	0.014
Calcium carbonate	3	----	1.200
Sodium chloride	1	----	----
Total	100	0.223	1.228

Ten milligrams of ferrie citrate and 1 c.c. of cod liver oil containing 250 vitamin D units were added to the above ration which was fed each rat every other day. An excess of food was kept in the cages at all times. A control group was fed the following diet which is higher in phosphorus and protein. The ferrie citrate and cod liver oil were added as in the low phosphorus diet. The meat powder was prepared from beef round from which the visible fat had been removed by cutting. The lean meat was ground, desiccated in vacuum at 56° C., and pulverized.

Ten disease-free white female rats from the Wistar strain and twenty-eight days old were fed the low phosphorus diet for one hundred and thirty-two days. An identical group was fed the diet higher in phosphorus. At the end of the feeding period the long bones were x-rayed, the gastrointestinal tract removed,

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TABLE II

FOOD	PER CENT IN RATION	PHOSPHORUS IN GM IN 100 GM OF RATION	CALCIUM IN GM IN 100 GM OF RATION
Corn	5	0.009	0.001
Oats	50	0.198	0.074
Meat powder	41	0.227	---
Calcium carbonate	3	---	1.200
Sodium chloride	1	---	---
Total	100	0.434	1.275

and the animals incinerated in an electric furnace. The ash was analyzed for calcium by the volumetric oxalate method and for phosphorus by the amino naphthol sulphonic method.

RESULTS

The average of the percentage of calcium in the ash of the animals fed the low phosphorus diet was 32 as compared to 40 in those fed the diet higher in phosphorus. The average of the percentage of phosphorus in animals fed the low phosphorus diet was 12 as compared to 6 found in those fed the higher phosphorus diet.

TABLE III
ANALYSIS OF RATS FED ON LOW PHOSPHORUS DIET

RAT	WEIGHT AT END OF EXPERIMENT IN GM	WEIGHT OF ASH IN GM	WEIGHT OF CALCIUM IN GM	PER CENT OF CALCIUM IN ASH	WEIGHT OF PHOSPHORUS IN GM	PER CENT OF PHOSPHORUS IN ASH
A	31	15.0	0.490	32	0.100	13
B	32	1.50	0.496	32	0.195	13
C	36	1.321	0.426	28	0.211	14
D	26	1.460	0.467	32	0.205	14
E	48	19.0	0.618	32	0.190	10
F	56	1.891	0.658	25	0.200	11
G	26	1.491	0.477	32	0.189	13
H	22	1.611	0.516	32	0.190	12
I	30	1.650	0.561	33	0.205	12
J	30	1.550	0.527	34	0.202	13
Average				32		12

TABLE IV
ANALYSIS OF RATS FED ON HIGH PHOSPHORUS DIET

RAT	WEIGHT AT END OF EXPERIMENT IN GM	WEIGHT OF ASH IN GM	WEIGHT OF CALCIUM IN GM	PER CENT OF CALCIUM IN ASH	WEIGHT OF PHOSPHORUS IN GM	PER CENT OF PHOSPHORUS IN ASH
AA	160	5.980	2.312	39	0.426	7
BB	168	5.912	2.306	39	0.421	7
CC	212	6.981	2.722	39	0.421	6
DD	172	6.660	2.600	39	0.480	7
EE	188	6.256	2.507	40	0.387	6
FF	200	8.351	3.507	42	0.414	5
GG	220	7.696	3.309	43	0.400	5
HH	212	7.760	2.949	38	0.467	6
II	172	6.542	2.548	39	0.400	6
JJ	180	6.731	2.469	39	0.392	6
Average				40		6

The blood counts and hemoglobin determinations by the Sahli method on all of the animals were normal at the end of the feeding period.

X-ray pictures taken of the long bones of the animals that were fed the low phosphorus diet were typical of rachitic bones. The bones bowed on pressure, the epiphyseal line was jagged and irregular, and there was a marked diminution in the calcification at the epiphysis. X-ray pictures taken of the long bones of animals fed high phosphorus diets were normal.

CONCLUSIONS

A diet low in phosphorus and protein, adequate in calcium and vitamin D, but with a calcium to phosphorus ratio of 5.5 to 1 will produce rickets when fed to rats. When the ratio is lowered to 3 to 1 by raising the phosphorus content by adding meat, the bones develop normally.

It will be noted that the percentage of phosphorus in the bones of the rachitic rat is higher and the calcium lower than that in the bones of the normal animal. This difference in composition is probably due to a higher percentage of the calcium present as the carbonate in the normal than in the rachitic bones.

V. NONRENAL AZOTEMIA*

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SINCE our report¹ in 1935, of various clinical conditions in which high blood urea values arise and in which there is no evidence of chronic glomerulonephritis, a number of other conditions have come under our observation. A number of important papers have also appeared which bear directly or indirectly on the problem of hyperazotemia of nonrenal origin.

In the discussion of our earlier presentation, we made an analysis of the various states with which high blood urea is associated and found that there was one large group in which the majority of clinical cases could be placed and concerning which there was a considerable amount of experimental data. This type which others had designated hypochloremic uremia or "Azotemie par manque de sel" appears to be closely connected with a severe, but reversible change in the salt and water metabolism of the organism, and proved to be the type which responds favorably to salt and water administration. Recently McCance² has produced a similar clinical condition by salt deprivation alone through salt-free diet combined with sweating.

There are other states of nonrenal azotemia that are not conditioned by demonstrable loss of water or electrolytes. There is a possibility that such a state may reflect some internal metabolic derangement which at present is not

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readily discernible. Thus, the injection of histamine into animals produces an increase in nitrogen in the plasma with a drop in the chlorides. There is no evidence of external loss of water and electrolytes through known channels, such as the stomach, the intestines, or the skin.²

Indeed, we called attention to the many points of similarity, both chemical and clinical, between the hypochloremic states and the crises of Addison's disease.¹ In more recent experimental work, Wohl, Buins, and Clark found morphologic alterations in adrenal glands of dogs, with hypochloremia resulting from experimental high intestinal obstruction.

With replacement of large quantities of normal salt solution (400 cc daily) without cortin or with smaller amounts of normal salt solution (200 cc daily) plus cortin, the clinical and biochemical signs of the toxemia abated,

TABLE I
CLINICAL CONDITIONS OF NONRENAL AZOTEMIA

Dehydration from
Loss of gastrointestinal secretions
a Cancer of stomach
b Pylorospasm
c Hyperemesis gravidarum
d Gastric tetany
e Alkalosis in alkaline treatment of peptic ulcer
f Acute intestinal obstruction
g Self treatment with duodenal tube
h Diarrhea
Loss of extracellular fluid
a Profuse sweating from exposure to extreme heat
b Diabetes mellitus
c Postoperative
d Therapeutic purposes: limitation of fluid (epilepsy)
Nonobvious loss of fluids and electrolytes
a Burns
b Histamine shock and other shocklike conditions
c Adrenal insufficiency

and the adrenal glands remained intact^{4, 5} (Fig. 1) (see Charts 1, 2). The various symptoms of cortico-adrenal insufficiency were explained by Zwemer and Tuszowski in terms "of a disturbance of cortico-adrenal potassium interrelations."⁶ Seudder, Zwemer, and Tuszowski fortified our contention that acute intestinal obstruction and adrenal insufficiency have many features in common, by demonstrating high blood potassium values in acute intestinal obstruction.⁷ The report of Harrop and his associates⁸ on the salt water hormone of the adrenals is provocative of the thought that a functional and temporary derangement of the adrenals may be associated fundamentally with some states of nonnephritic urea increases under discussion, the latter eventuating from fluid and base alterations between the extracellular tissues, the tissue cells, and the blood. The appearance of a high figure of blood urea nitrogen in any clinical case is properly regarded as pointing first to renal damage, especially if to this is added the presence of albumin and casts, as well as the manifestations of weakness or vertigo, confusion, or stupor. With our present knowledge, however, the possibility of other conditions causing this state of affairs ought to

make the physician complete the picture of Bright's disease before he consigns his patient to that sort of prognostic limbo. The blood pressure, the eye-ground pattern, the specific gravity of the urine, the kidney function tests, and the history of a previous illness leading to kidney disease, when kept in mind, will aid in differentiating true uremia from nonrenal hyperazotemia. The correct and early diagnosis may mean not only rapid clearing of toxic symptoms by intravenous saline administration, but may prevent the patient from going into deep coma and possibly death. We stressed in our first report, dehydration as the common denominator of the azotemia and hypochloremia and felt that some of the cases with obvious loss of secretions from the gastrointestinal tract,

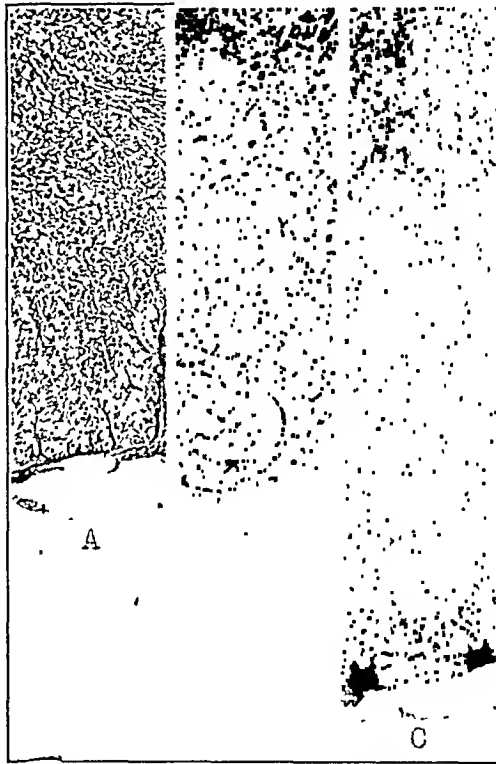


Fig. 1.—Low power photomicrograph of adrenal cortex in experimental high intestinal obstruction (all photomicrographs made at the same magnitude). A, extreme lipid exhaustion; zona glomerulosa "moth eaten" with rupture of cell walls; zona fasciculata: cytoplasm granular, apparently devoid of lipid; B, partial lipid depletion (dog receiving insufficient quantities of salt solution); zona glomerulosa filled with lipid; cell walls preserved; zona fasciculata only partially depleted; cytoplasm contains lipid droplets; C, no evidence of lipid depletion; "storage phase" (dog receiving sufficient quantities of salt solution); zona glomerulosa and zona fasciculata well filled with lipid; cells foamy; note increased width of cortex as compared with A and B.

forced limitation of fluid and salt intake for therapeutic purposes (epilepsy), or postoperatively belong to this group; but we had no case like the following, the pathogenesis of which remains obscure.

CASE 1.—A colored boy, ten years old, was admitted to the Philadelphia General Hospital on March 14, 1936, with a history of weakness, "inward fever" cough, abdominal pain, anorexia, and constipation. He had been in good health up to Feb. 29, 1936, when there was gradual and progressively increasing weakness and loss of energy. He developed "some

fever," with a cough afterward. Anorexia and constipation were profound from the beginning, but there had been no vomiting until five days after he was forced to bed because of weakness. Examination disclosed an emaciated, weak, and apathetic colored boy, tossing about in bed. There was no vomiting, except once when milk feeding was attempted. The blood pressure was 80 systolic; diastolic was unobtainable. Lungs were clear; heart sounds distant and slow, and the abdomen was scaphoid. There were no masses or tenderness. The spleen was not palpable. The temperature range was normal.

A diagnosis of marasmus, dehydration, and gastrointestinal upset was made. The laboratory studies were as follows: Urine: acid, specific gravity 1.019. Albumin and sugar negative. No acetone, no casts were found, leucocytes and erythrocytes, occasional. Blood count: erythrocytes 4,460,000, leucocytes 26,000, hemoglobin 13.6 gm.; polymorphonuclears 82 per cent, lymphocytes 18 per cent. Blood: Kahn negative; blood sugar 160.0; urea nitrogen 260 mg. per cent. On March 16, 1936: blood sugar 121.0 and urea nitrogen 170 mg. per cent. On March 17, 1936: creatinine 5.0. Blood chlorides 694 mg per cent; blood CO_2 , 41 volumes per cent. The boy was placed on continuous hypodermoclysis of 3 per cent glucose in normal salt solution and began to improve at once. In four days his blood pressure was 102/60, but he had a bradycardia of 58 with some extrasystoles. On March 23, 1936, the blood urea nitrogen was 20 mg. per cent; the blood chlorides were 610 mg per cent and the patient

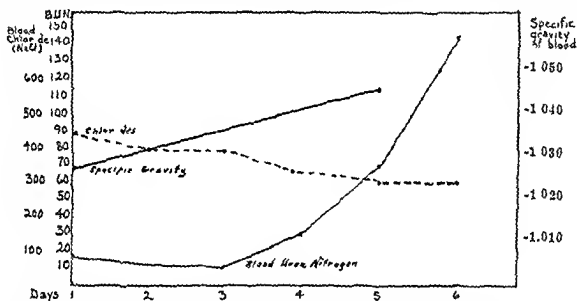


Chart 1—Blood findings in experimental high intestinal obstruction showing marked rise in urea nitrogen and specific gravity of blood, decrease in blood chlorides (Wohl, Burns, and Pfeiffer²)

was entirely better, happy, and eating heartily. The spinal fluid was negative, except for a chloride content of 956 mg. per cent during salt administration. The urea clearance on March 23, 1936, was 59 per cent the first hour, 72 per cent the second hour.

This boy returned for examination on May 22, 1936, after a healthy interim and his urinalysis was perfectly normal (seven specimens were examined). The blood urea nitrogen ranged from 21 to 10 mg. per cent. The urea clearance was 165 per cent, first hour clearance, 131 per cent second hour. The phenol sulphonephthalein was 73 per cent in one hour. The spinal fluid was negative. Physical examination, x-ray studies of chest and gastrointestinal tract furnished no clue as to his former illness.

Comments.—The prolonged malnutrition with its accompanying decrease in the blood volume would appear to be the probable underlying basis for the increase in blood urea. There must have been an undue loss of intracellular fluid with an acidosis. The CO_2 was 41 volumes per cent. It is worthy to note that at the time the blood urea had arisen, the blood chlorides remained at a normal level. This would strengthen the point of view that the hyperazotemia may occur without the hyponchloremia. This is contrary to Blum's contention that hyponchloremia is primary and the azotemia compensates for the

low osmotic pressure of body fluid resulting from loss of electrolytes. Furthermore, from a practical therapeutic consideration, it should be stressed that not all the patients with hyperazotemia should be given salt solution. In patients who show normal blood chlorides, dextrose in distilled water is preferable. This procedure may avert subsequent edema and irritative cerebral phenomena (hydration of the brain) if excessive salt solution is given in cases with normal blood chlorides. On the other hand, this boy's condition might conceivably have been an acute but temporary functional derangement of the adrenals, with alteration of the intra- and extracellular fluid and electrolyte distribution. Whether an abnormal release of potassium might account for the symptoms of low blood pressure, asthenia, and anorexia cannot be definitely stated. Unfortunately, potassium studies were not done in this case.

It is not generally appreciated that increased blood urea nitrogen figures occur in gastrointestinal hemorrhages, although, recently, it has been commented upon in the literature.^{9, 10, 15, 16} Of course, it has, for a long time, been known that experimentally sudden loss of blood in itself will be followed by an

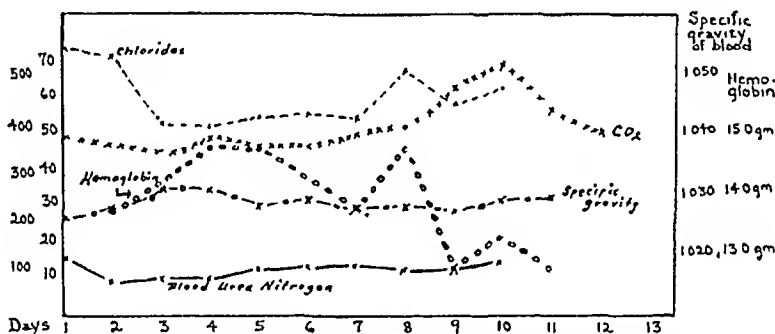


Chart 2.—Experimental high intestinal obstruction treated with extract of adrenal cortex and salt solution. Note the insignificant change in urea nitrogen and specific gravity of blood; blood chlorides are only slightly decreased. (Wohl, Burns, and Pfeiffer.⁵)

increase of blood nonprotein nitrogen values.^{11, 12} Likewise, shock due to wounds will show a similar picture.^{13, 14} The following cases illustrate azotemia due to acute gastrointestinal hemorrhage.

CASE 2.—N. D., a white man, fifty years old, who for at least two years prior to admission to the Philadelphia General Hospital had a typical peptic ulcer history with pain relief from alkali powders. About a week prior to admission to the hospital, he vomited undigested blood (about one quart of material in all) and passed tarry stools. Examination showed an Italian man with a warm, dry, and faintly icteroid skin, who appeared acutely ill, but was not complaining of any pain. The blood pressure was 140 systolic, 80 diastolic. Physical examination was normal so far as revealing any pertinent signs of the underlying condition was concerned. The man was diagnosed as a bleeding peptic ulcer case. The blood count showed a rather marked secondary anemia, and the blood urea nitrogen was 40 mg. per cent on June 6, 1936. Another blood specimen taken on June 8, 1936, showed a urea nitrogen of 90 mg. per cent. Because of the rising blood urea nitrogen, secondary anemia and the presence of casts in the urine, a note by the interne suggested reconsidering the diagnosis, although he added that the clinical state did not fit in with the picture of chronic glomerulonephritis.

By June 9, 1936, the blood pressure was 116 systolic, 74 diastolic. The heart and lungs were negative. On a Sippy diet, the man continued to improve. By June 11, 1936, the blood pressure was 130 systolic, 74 diastolic, and he was considered fit under cyclopropane anesthesia for operation, which was performed June 11, 1936, and a duodenal ulcer was found.

By June 10, 1936, the patient's blood urea nitrogen was 15 mg per cent, and on the day of the operation it was down to 12 mg per cent. His convalescence was uneventful from every standpoint. The diagnosis of bleeding into the bowel remained the chief element associated with the cause for his admission and the immediate forerunner, if not the actual cause, of the azotemia.

CASE 3—Mrs. D. B., a white woman, aged forty-nine years, admitted to Temple University Hospital with a chief complaint of fainting and weakness with vomiting of black material and passage of "pitch black stools" the day before admission Dec. 1, 1936. The vomiting was reported once before admission.

The past medical history was irrelevant, including the gastrointestinal tract which was entirely negative up to the present illness. The patient was noted as one who was accustomed to endure hardships to work hard and to bear many children. The physical examination being negative, a diagnosis of bleeding duodenal ulcer was made, based on the history. The blood pressure was 90 systolic, 65 diastolic. The blood count on admission was erythrocytes 3,180,000, leucocytes 18,400, polymorphonuclears 86 per cent, lymphocytes 16 per cent. The urine showed a specific gravity 1.017, a trace of albumin with negative microscopic findings. The blood chemistry reported on the day after admission, Dec. 2, 1936, showed the urea nitrogen to be 88 mg per cent and the blood chlorides to be 524 mg per cent. The patient was given several blood transfusions. She improved markedly in general and so far as the blood count and the blood chemistry were concerned, the latter in three days (by Dec. 5, 1936) was decreased to 26 mg per cent urea nitrogen. Her blood pressure on Dec. 7, 1936, was 190 systolic over 100 diastolic and the eye ground examination revealed a severe grade of arteriosclerosis. Occult blood was still present in the stool on Dec. 30, 1936, and continued to the time of discharge on Jan. 19, 1937, although the patient felt well, and the erythrocyte count was 4,450,000. She had been put on a Sippy diet. Her blood pressure on discharge Jan. 19, 1937, was 240 systolic over 136 diastolic and she was put down definitely as a case of essential hypertension. Her stools remained positive for occult blood throughout her stay in the hospital, although proctoscopic examination revealed no signs of bleeding from hemorrhoids or other disease of the lower bowel. It should be added that an x-ray series on Dec. 15, 1936, was reported as definitely indicating a duodenal ulcer on the lesser curvature of the first portion of the duodenum.

Comments—These two cases parallel closely those reported by Sueie¹⁵ and Ingegno¹⁰ in that high blood urea values are observed within a short time after obvious bleeding into the gastrointestinal tract. The hyperazotemia declines promptly on cessation of the bleeding. The persistence of a positive occult blood test in the stools in the last case is at variance with the impression of Ingegno, that continued bleeding results in persistence of elevated urea values and which, he, therefore, assumes to be of great prognostic value. The amount of actual bleeding in this case, it is true, may be questioned, as the blood count and other signs of hemorrhage did not speak for continued loss of appreciable quantities of blood into the bowel. It has been suggested that the elevation in blood urea may be due to increased liberation of the protein of the blood into the intestines and to resorption of blood proteins from the intestinal tract.

If this explanation approximates the truth, then one would expect at least some elevation of the blood urea in patients with ruptured ectopic pregnancy. With this point in view, we reviewed twenty case histories of patients who were operated upon at the Philadelphia General Hospital in the last two years (Table II). All the urea nitrogen values, it will be seen, were well within normal range.

It would seem, therefore, that the azotemia in hemorrhage is probably not conditioned by the resorption of blood. The loss of plasma and electrolytes through the intestinal tract would appear to be the more correct explanation. Continental writers refer to the condition of hyperazotemia of gastrointestinal hemorrhage as "exhaustio virium."¹⁸ If it is unrecognized and not treated early by sufficient amounts of sodium chloride solution, the patient usually dies. Death in such instances, as generally held, is due to extrarenal uremia rather than to the severe anemia.

TABLE II
BLOOD FINDINGS IN CASES OF RUPTURED ECTOPIC PREGNANCY

CASE NO.	PROBABLE DATE OF RUPTURE	OPERATION DATE	UREA	BLOOD TAKEN DATE	ERYTHROCYTES	LEUCOCYTES	WASSERMANN
1	Old	2/ 2/36	13.0		4.3	9,600	++++
2	2/ 2/36	2/ 6/36	10.0	2/ 8/36			
3	11/12/36	11/23/36	10.0	11/19/36	4.1	10,600	Neg.
4	10/30/36	11/23/36	11.0	11/10/36	2.5	20,550	+
5	11/27/36	12/14/36	10.0	12/ 3/36	2.6	5,120	++++
6	?Bleeding	8/27/36	10.0	8/24/36	3.5	3,560	++++
7	6/16/36	6/17/36	14.0	6/18/36	2.4	10,300	
8	4/11/36	4/12/36	14.0	4/14/36	4.1	13,950	Neg.
9	7/17/36	8/27/36	10.0	8/24/36	3.5	12,000	++
10	12/ 2/36	12/15/36	10.0	12/ 3/36	2.6	5,150	++++
11	11/ 9/36	11/23/36	11.0	11/10/36	2.4	17,900	++++
12	1/23/36	1/23/36	11.0	1/27/36	2.8	14,800	Neg.
13	3/11/36	3/12/36	15.0	3/16/36	3.6	12,550	Neg.
14	2/ 4/37	2/ 8/37	8.0	2/ 8/37	2.5	14,150	Neg.
15	2/ 6/37	2/ 6/37	10.0	2/ 6/37	4.1	14,500	Neg.
16	?	9/15/36	9.0	9/ 8/37	3.5	11,900	Neg.
17	10/30/36	11/ 7/36	8.0	11/ 9/36	2.5	20,500	+
18	6/ 3/36	6/ 3/36	13.0	6/ 3/36	3.6	7,200	Neg.
19	1/31/37	2/19/37	12.0	2/20/37	4.3	8,600	Neg.
20	1/27/37	1/27/37	15.0	1/23/37	2.9	17,900	Neg.

A very interesting case of a type not hitherto reported in the literature is that of unresolved bronchopneumonia treated by x-ray.

CASE 4.—A white man, E. W., aged 50 years, admitted to the Philadelphia General Hospital, Feb. 16, 1936, for cough and shortness of breath; the physical signs as well as the x-ray the following day indicated a confluent bronchopneumonia. The day after admission his blood urea nitrogen was 21 mg. per cent. On March 3, 1936, he began to have x-ray treatments of his lung on account of failure to resolve. By March 6, 1936, his blood urea nitrogen had risen to 105 mg. per cent. The urinalysis was acid; specific gravity 1.015; albumin 0; sugar 0. Microscopic examination negative. A Mosenthal test showed a specific gravity of 1.010 to 1.017. Blood count was 3,500,000; the blood Wassermann was negative. By March 16, 1936, his urea nitrogen was 36 mg. per cent and his blood chlorides were 605 mg. per cent. The patient developed erysipelas during convalescence, and on his return from the contagious disease hospital to which he had been transferred, he had a normal urinalysis and a blood count of 3,670,000 erythrocytes, 9,850 white blood cells, and a normal differential. The blood urea nitrogen was 20 mg. per cent.

Another similar case came under our observation at the Philadelphia General Hospital (service of Dr. William Egbert Robertson) of a young woman, thirty years old, who received x-ray treatments of her lung for an unresolved lobar pneumonia. Prior to the irradiation, the blood urea was 12 mg. per cent. Following the x-ray therapy, the blood urea had risen to 90 mg. per cent. The urinalysis, the Mosenthal test, and the eye-ground examination failed to implicate the kidneys as the cause of the azotemia.

A rise in blood urea nitrogen during the course of malarial therapy of dementia paralytica has been of frequent occurrence in patients at the Philadelphia General Hospital. In view of our interest in nonrenal azotemia, it appeared desirable to make a more critical study of these changes.

The records of two hundred and fifty cases of paresis admitted to the Philadelphia General Hospital between the years 1928 and 1937 were examined to determine the normal blood urea nitrogen for such a group, and also to note any changes in this finding that might occur following fever therapy, with particular reference to malaria. It should be noted here that many of these cases showed various other forms of syphilis or complicating systemic diseases.

All but sixteen of these 250 cases had a blood urea nitrogen of 20 mg per cent or under, the average being 14 mg per cent. Twenty milligrams per cent was considered as the top normal.

Ninety of the cases showing normal blood urea nitrogen figures were given a course of malarial therapy, averaging ten elevations of temperature. Of this group 35 cases (37.7 per cent) had urea nitrogen increases of varying degree, up to 110 mg per cent, the average being 39 mg per cent. Determinations of urea nitrogen were usually obtained biweekly in every case of paresis so treated. In analyzing these cases, it was observed that the urea rise might occur at the beginning, middle, or end of treatment, and, therefore, no prediction could be made of this possibility in any given case. It was concluded, however, that increases above 40 mg per cent were of bad prognosis inasmuch as of seven of the patients who had such rise, three (43.0 per cent) died, while of the twenty-eight others whose rise in blood urea nitrogen was less than 40 mg per cent, only five (17.5 per cent) died. No changes were observed in the plasma chlorides. In a few cases where plasma chlorides were determined at the time of a sudden urea rise, no abnormal depression of the former was noted. Other workers have reported some reductions in chlorides in patients undergoing malarial therapy.¹

Comments—Blood urea nitrogen frequently, though by no means always, is increased during the course of malarial therapy of paresis.

SUMMARY AND CONCLUSION

It is still not generally appreciated that high blood urea nitrogen values are found in many diverse clinical states other than chronic glomerular nephritis. In the majority of instances, the clinical and biochemical changes of the blood reflect loss of electrolytes and fluid through secretions from the gastrointestinal tract.

There are, however, other clinical states in which there is no such obvious loss of electrolytes and fluids. A series of such cases is described. It is to be stressed that the group of conditions associated with hyperazotemia and hypochloremia bear certain clinical and biochemical similarities. Likewise, they have many features in common with adrenal insufficiency, as was pointed out in our earlier publication. In the light of the recent work of Harrop on the salt water hormone of the adrenal cortex, which controls sodium metabolism and our findings of morphologic changes in the adrenals in hyperazotemia and

hypochloremia of high intestinal obstruction, it is strongly suggestive that the pathogenesis of the many similar cases of disturbed electrolyte and water metabolism, with associated hyperazotemia and hypochloremia, may find its proper explanation in a cortico adrenal insufficiency.

A special study was made of cases of dementia paralytica treated with malaria. In 37.7 per cent of such patients, there was demonstrable a nonrenal azotemia with an average of 39 and a maximum of 110 mg. per 100 c.c. blood.

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THE SOURCE OF URINARY INDOL*

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INDOL was recognized as early as 1875 as a product of bacterial decomposition of proteins. In 1901, Hopkins and Cole discovered tryptophan, and in 1903, these same authors demonstrated that tryptophan is the source of bacterially formed indol. It has been shown that tryptophan is the only constituent of the protein molecule which can be transformed into indol by bacteria.¹

Since practically all proteins contain tryptophan and since the gastrointestinal tract is heavily laden with the types of organisms which are most active in indol formation, it might be expected that indol would be formed in the intestine and pass into the blood stream. Under normal conditions this indol does not appear in the free state in the urine because of the efficient detoxifying mechanism which is available. Free indol, however, has been reported as present in the urine in various pathologic conditions.

The appearance of free indol in the urine might indicate a failure of the detoxifying mechanism which is presumably mainly localized in the liver. On that basis its determination in the urine could serve as a test for liver dysfunction. With this in mind Vaughan examined the urines from a series of patients suffering from various pathologic conditions of the liver and also from patients with severe putrefaction and intestinal stasis.² He was not able to find indol in any of the urines tested. The method employed was to precipitate bilirubin with barium chloride, extract the filtrate with petroleum ether, and test with Ehrlich's reagent.

Forbes and Neale³ using a different analytical method which involved two steam distillations and testing the final distillate with sodium benaphthoquinone sulfonate, examined urines from patients presenting a variety of pathologic conditions. No indol was found in the urine from normal individuals, but it was found in significant amounts in many cases of chronic arthritis, pellagra, diabetes mellitus, tuberculosis, toxemia of pregnancy, congestive heart failure, and lobar pneumonia. Amounts of 0.05 mg and more per liter were considered significant.

More recently Vaughan⁴ has advanced the argument that the positive tests may in many cases be explained by bacterial contamination.

We have employed the method of Forbes and Neale and have confirmed the statement that the urine of certain individuals gives a positive test for indol. It is with a possible explanation of these findings and the action of pure cultures of bacteria on sterile urine filtrates that we shall deal in this communication. Our experiments were carried out in the summer of 1935, but publica-

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tion was delayed pending further work which is now in progress. Recent interest in the subject prompts the authors to report the results of the first part of the investigation at this time.

As indicated above we feel that indol in the body is always a product of bacterial action. For the ultimate source it only remains to determine the locus of indol-producing organisms whether they be in the intestinal tract, some focus of infection, or in the urinary tract. A knowledge of the type of organism is very important since the specificity for indol production varies greatly. It is also of paramount importance to know whether similar compounds other than tryptophan itself can serve as the mother substance.

Indol has been demonstrated by Ehrlich's method as a product of the action of *E. coli*, *Proteus vulgaris*, pseudodysentery, and true vibrios.⁵ *E. coli* is most vigorous in its attack on tryptophan,⁶ and the indol so formed is not decomposed by the bacteria—in fact, indol in a concentration of 1:1500 has bacteriostatic qualities against *E. coli*.⁷ These organisms are abundant in the intestinal tract. It has been suggested that they may pass rather readily into the blood stream, and it is not inconceivable that the average urine specimen might contain them, particularly if the conditions in the urine were favorable for viability. *E. coli* has long been incriminated as an infecting organism of the urinary tract. Traut and Kuder reported this organism to be present in 90 per cent of cases of urinary infections during pregnancy.⁸

Woods⁹ has investigated the action of *E. coli* on compounds other than tryptophan (α-amino β-indol propionic acid). No indol was formed, under aerobic conditions from β-indol aldehyde, β-indol carboxylic acid, β-indol acetic acid, β-indol propionic acid, or β-indol acrylic acid. There was 10 per cent production from β-indol pyruvic acid when ammonia was present. In our experience, when certain strains of these organisms are grown in sterile urine filtrates, the reaction becomes alkaline due, no doubt, to the formation of ammonia. Woods⁹ has also shown that under anaerobic conditions *E. coli* converts tryptophan to indol propionic acid. In both instances the rate of indol formation was proportional to the amount of tryptophan present.

Saito¹⁰ has reported the production of indol by the action of *E. coli* on d-indol lactic acid. The l-isomer under the same conditions did not form indol. Indol pyruvic acid also gave rise to indol but required fresh cultures of the organism. A medium containing asparagin and inorganic salts was employed in these studies.

It is evident, therefore, that if urine is contaminated with *E. coli* and contains tryptophan, β-indol pyruvic acid, or d-indol lactic acid, indol will be formed during incubation, provided conditions are optimum. With this in mind, we have attempted to determine whether this might be the explanation of the positive indol tests which we and others have obtained on specimens of urine.

It was first necessary to deal with sterile urine filtrates in order to be certain that no contaminants were present. This was particularly true since Forbes and Neale invariably used morning urine samples, which had been in the bladder for several hours, and apparently took no precaution against contamination. We felt that perhaps the patients' urinary tracts were infected with *E. coli* or that the bacteria gained entrance after the urine was passed. If the determinations

were not made promptly or if the bladder contained organisms, it might explain the positive tests of Forbes and Neale as will be shown presently

In the experiments three pure cultures of *E. coli* were used two of which were isolated from catheterized specimens from patients suffering with infections of the urinary tract. The third came from a sample of buttermilk obtained at a local dairy. All three were vigorous indol producers from sterile media containing tryptophan.

Normal urines were passed through sterile filtration systems each specimen separately, the sterility of the filtrate determined and the sterile filtrates inoculated from one of the pure cultures of *E. coli*. These inoculated filtrates were incubated for varying periods at 37° C. Control filtrates were retained in each experiment. Distillations were carried out on the filtrates according to the method outlined by Forbes and Neale. The results on the control filtrates were compared with the original distillation of the urine, performed at the time the specimen was received and in most cases within a few minutes after collection. Typical experiments taken from a rather large number are shown in Table I.

TABLE I
URINE FILTRATE STUDIES

SPECIMEN	CULTURE USED	ORIGINAL	INDOL IN	
			SEEDED FILTRATE	CONTROL FILTRATE
HEC	B	trace	+ 96 hr	trace 96 hr
HEC	A	trace	+ 72 hr	trace 72 hr
10	7	trace	+ 6 hr	trace 36 hr
			+ 36 hr	
9	7	-	+ 6 hr	- 24 hr
			+ 24 hr	
13	A	-	+ 24 hr	- 24 hr
5	A	-	+ 4 hr	- 75 hr
			+ 8 hr	
			+ 24 hr	
			+ 75 hr	

trace = less than 0.5 mg per liter + = more than 0.5 mg per liter

The results of the entire series of experiments may be summarized as follows:

1. The organisms grew rapidly in the urine filtrates. The formation of a pellicle was noted in some of the cultures.
2. The pH increased in proportion to the time of incubation as is seen in contaminated urine which is allowed to stand.
3. None of the controls contained more indol than the original urine specimen.
4. All inoculated filtrates were strongly positive to the test for indol.
5. The indol test on inoculated specimens became positive within a short time (five to six hours) and further incubation did not increase the amount of indol formed.

We have noted, as did Vaughan, that specimens which were at first negative for indol became positive on standing. For example, a sample which did not give an indol test was allowed to stand for ninety-six hours, at which time it had become positive.

Cole¹ states that indol is not formed from tryptophan by *E. coli* in the presence of glucose. As an added check on our conditions, we have carried out a few experiments in which glucose was added to the urine before filtration. Typical results are given in Table II. It will be noted that indol was not formed in any of the filtrates which contained 0.2 per cent or more of glucose. One would not, therefore, expect to find indol in diabetic urines containing this much glucose, unless it is formed elsewhere in the body and is present in the urine merely because it has escaped conjugation. Forbes and Neale² report positive tests on diabetic urines, but they do not state how much glucose the urine contained, or whether that particular sample contained any at all. We have not as yet had an opportunity to examine urines from patients with diabetes.

TABLE II
URINE FILTRATE STUDIES

BOTTLE	CULTURE	TREATMENT	RESULT
1.	7	0.2% glucoso	negative indol test
2.	7		positive indol test
3. (ontrol)	uninoculated		negative indol test
All bottles incubated 20 hours at 25° C.			
BOTTLE	CULTURE	TREATMENT	RESULT
1.	7	0.3% glucoso	negative indol test
2.	7		positive indol test
3. (ontrol)	uninoculated		negative indol test
All bottles incubated 5 hours at 28° C.			

There is also the possibility that the indol obtained from the inoculated filtrates in which the organisms were growing rapidly was within the bacterial cells themselves, and not directly related to any substrate in the urine specimens. To eliminate this possible source of error, we have performed the following test. Culture A was grown on agar for forty-eight hours, washed off with isotonic salt solution, and diluted to 100 c.c. There were twenty thousand million organisms per cubic centimeter. This suspension, when used in the regular distillation, gave a negative indol test.

These experiments indicate, we believe, that normal urine contains a substrate from which *E. coli* can produce indol. The fact that we noted early indol production and that further incubation produced no increase indicates that the substance is present in small, but rather constant, amount. The short time interval before indol becomes detectable argues for the substrate being a simple compound, since apparently combined tryptophan must first be released from its combination. The formation of the necessary enzymes takes time, and indol does not appear for a considerable time; a much longer time than the five to six hours, which we have noted in our experiments.

This is a confirmation under somewhat different conditions, using a different-analytical method, of the work of Vaughan.

According to the results of other investigators discussed above, this substrate may be either tryptophan, indol pyruvic acid, or d-indol lactic acid. Isolation in pure form and chemical identification will, of course, be the final

proof of whether the material is one of the compounds mentioned, or some indol derivative whose behavior with *E. coli* has not yet been studied. Further investigation along this line is now in progress.

SUMMARY

1 Free indol has been found in some urine samples and was invariably found in sterile urine filtrates which had been inoculated with pure strains of *E. coli*.

2 The addition of glucose inhibits this indol formation.

3 Studies of sterile filtrates point to the possibility of the presence of some indol precursor in normal urine.

4 It is suggested that this precursor is tryptophan or some closely allied indol derivative.

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STUDIES ON MENINGOCOCCUS BROTH FILTRATES*

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THE presence of a soluble exotoxin in broth filtrates of meningococci, and the use of this toxin in skin tests on human subjects was reported by Herold and Traut¹ and by Ferry, Norton, and Steele.² Ferry³ tested a series of children and attempted to immunize the positive reactors by graded injections of toxin. The same tendency to associate susceptibility to infection and a positive skin reaction to this toxin was shown by Kuhns,⁴ who found that in patients recovered from the disease the skin test was negative. This relationship, however, is by no means proved, as was pointed out by Maxcy.⁵ Most investigators, from Gordon⁶ to the present time, seem to doubt the

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existence of such an exotoxin, and maintain, as do Maegraith,⁷ and Malcolm and White,⁸ that toxic factors present in such filtrates may be products of autolysis of the bacterial cell. Rake⁹ also seems to favor this hypothesis.

The pathogenicity of endotoxins present in autolysates of meningococci for various laboratory animals, notably rabbits and mice, has often been demonstrated.¹¹ Malcolm and White⁸ noted that rabbits develop a tolerance or immunity to these substances if gradually increasing doses are employed. Experiments with exotoxin have been less common. Ferry¹⁰ reported that monkeys surviving the injection of this substance intraspinally were thereafter immune to infection with virulent meningococci. The greater part of his reported work with animals, however, deals with their passive protection by means of antitoxin, and not active immunization with the toxin. The present work was designed to investigate further the skin reactions of various groups of individuals to dilutions of meningococcus broth filtrates, and the immunizing action of these filtrates in mice.

The broth filtrate was prepared according to the directions in Ferry's original article.² Four strains of meningococci, belonging to Group I-III, which had been isolated from patients within the previous eight weeks, were used. As recommended by Kulms⁴ merthiolate 1:10,000 was added to the filtrate as a preservative. The skin testing material was made up in dilutions of 1:1,000 and 1:800 in saline, and a control provided consisting of Ferry's hormone broth in the same dilutions and containing the same proportion of merthiolate.

Two groups of normal controls were first tested by injecting 0.1 c.c. each of toxin and broth control intracutaneously in the skin of the forearm. The reactions were read at the end of twenty-four hours, and only those showing a zone of erythema greater than 1 cm. in diameter were considered positive. Twenty-two adults showed 86 per cent positive reactions, and 16 children gave 56 per cent positive reactions. Broth controls were uniformly negative, both in these and in subsequent experiments.

Twenty-four cases of meningococcus meningitis who had recovered within the past two years were tested. These individuals were clinically well at the time and varied in age from infancy to forty-five years. It was found that 76 per cent of these gave positive skin reactions, which is about what one would expect in a control group of comparable age distribution. Following this, 13 cases, ill or just recovering from meningococcus infection, were tested, and all but one were found to be skin test negative. However, when a series of cases sick with one of a number of other infections such as tuberculosis, rheumatic fever, scarlet fever, pneumonia, and undulant fever were tested, it was found that there was a great predominance of negative reactions. Of 34 such patients, only 9 or 26 per cent gave positive reactions, which is a marked reduction from the 74 per cent of the combined control groups. A comparison of the results is presented in Table I.

We attempted to correlate the presence of the organisms in an individual with a negative or a positive skin test. Throat cultures were obtained from

the 24 recovered cases mentioned above at the time the skin test was done, and smeared directly on warm North gelatin agar plates. Two carriers were discovered, both of whom gave negative intradermal reactions. One carrier, however, not in this series of recovered cases, was later discovered who had a strongly positive skin test. It appears, therefore, that in these few instances there is no evidence pointing to an association of the carrier state with a negative skin test. The occurrence of a positive skin test in one patient with meningococcus meningitis also speaks against any such association.

As can be seen in Table I, the group showing the lowest per cent of positive skin reactions is the sick meningococcus cases. The somewhat similar findings in the control group sick with other diseases make it seem likely that

TABLE I

GROUP	NUMBER TESTED	TOTAL	PER CENT POSITIVE	
Control adult	22	38	86	74
child	16		56	
Recovered cases	24		76	
Sick meningococcus cases	13		8	
Control sick cases	34		26	

these results can be explained as a nonspecific phenomenon. It is possible, however, that in some cases patients sick or recovering from meningococcus meningitis might contain antibodies in their serum capable of neutralizing the toxin of the skin test dose and so avoid any reaction. Serum from two such patients, removed on four occasions failed to have this effect either when mixed with the toxin *in vitro* before injection in four individuals, or when injected the day before at the site of the skin test. No neutralization of the skin test dose could be demonstrated in animals. Thirty rabbits gave uniformly positive skin tests, and remained positive even after 15 of these animals had received 3 or more injections of antimeningococcus serum, or had been immunized with suspensions of live organisms or intravenous toxin in large and repeated doses. Moreover, in three of the meningitis patients followed for two months after discharge, the skin test became positive. Hence it would seem that it is not the presence of specific infection nor the production of antibodies, but rather a temporarily altered reactivity of the skin at the time of any acute infection that is responsible for the negative skin tests in these patients.

To determine whether the filtrate had any immunizing effect against doses of virulent meningococci in white mice, a series of ten experiments were carried out, the number of mice in each ranging from 6 to 10, with an equal number of controls. One hundred fifty mice were used in all. The filtrate was injected intraperitoneally in doses of 0.5 cc at four day intervals the mice receiving 2 to 2.5 cc in all, while the control animals received the same doses of hormone broth. The injections of filtrate had no toxic effect. From ten to twenty two days after the last injection of filtrate or broth the mice were given intraperitoneally 0.5 cc of a heavy saline suspension of meningococci washed from North gelatin agar plates. The results in each of the

ten experiments are similar, the filtrate-treated mice were much less affected by the meningococcus suspensions. The combined results of eight experiments are summarized in Table II.

TABLE II

	NUMBER OF MICE	MORTALITY	CLINICALLY* SICK	MORBIDITY TOTAL PER CENT
Filtrate treated	46	5	3	17.3
Broth treated	42	25	13	90.4

*By "clinically sick" is meant marked ophthalmula, roughening of the fur, and prostration so that the animals do not move when prodded.

This protective effect of the filtrate was apparently temporary. As can be seen in Table III, two series of mice injected with organisms forty-two days after the last filtrate injection were not protected as completely.

TABLE III

	NUMBER OF MICE	MORTALITY	CLINICALLY SICK	MORBIDITY TOTAL PER CENT
Filtrate treated	16	11	6	68.9
Broth treated	13	12	1	100.0

Four mice that had recovered from an injection of live meningococci after treatment with filtrate were reinjected with meningococcus suspension sixty days later. Two animals died and two were sick, giving a total morbidity of 100 per cent.

These experiments suggest that broth filtrates of meningococci have some active immunizing action in white mice against suspensions of the live organisms. Since in skin tests on human beings, these filtrates seemed to be non-specific, we believe that different substances are responsible for the skin reactions and the effects in mice. We realize that this series is by no means conclusive and requires further study.

Very little is known about the composition of filtrates of meningococcus broth cultures, and therefore the results obtained in skin tests or animal experiments may be due to any one of many hypothetical products. Rake⁹ has made definite advances in separation and analysis of the antigenic complex of the meningococcus, but the substances that he studied were obtained from a much older culture than we used here. Ferry³ and Kuhns⁴ have shown that autolysates from meningococci failed to give skin reactions comparable to those obtained with broth filtrates, and have thus supported their belief that the filtrates contain a true toxin and not merely products of autolysis. We repeated these experiments as follows: Saline suspensions of meningococci grown on solid media for twenty-four hours were spun down, the supernatant fluid discarded, and the sediment placed in the ice box till cultures were sterile. The organisms were then resuspended in hormone broth, the suspension being made equal in density to a four-day growth of the same strain of meningococci in hormone broth. The living culture was then filtered, while the suspension of killed organisms was placed in the incubator for four days before being filtered. Merthiolate 1:10,000 was added to both products after filtering. Parallel skin tests with similar dilutions of the two products showed a good positive reaction with the broth culture filtrate, but a negative result with the autolysate. Such results,

obtained by various workers, though suggestive, are not final evidence in favor of Ferry's hypothesis of an exotoxin, since it is impossible to exactly reproduce the conditions, enzymatic and otherwise, present in a live broth culture. Although it seems unlikely that the substance responsible for the skin test is a product of autolysis, the possibility remains that it may result from the decomposition of the broth medium by the action of growing meningococci.

Through the kindness of Dr. Rake, we obtained some purified specific Type I polysaccharide, and were able to compare known dilutions of this substance with various dilutions of autolysates and filtrates by means of precipitin tests with specific Type I rabbit serum. This serum had previously been absorbed with Type II organisms. The precipitin tests were carried out in the manner described by Rake and Scherp.⁹ All meningococcus broth filtrates used in our experiments gave positive precipitin tests, demonstrating the presence of the type specific polysaccharide. There were also detectable amounts of the polysaccharide in some, but not all, of the preparations of autolysate. It would seem, then, that Ferry's broth filtrate contains various, little understood, substances, among them at least one substance identified as the type specific polysaccharide of the meningococcus isolated by Rake and Scherp. The substance responsible for the skin reactions and its mode of production still eludes definite analysis.

CONCLUSIONS

(1) Skin reactions using dilutions of meningococcus broth filtrates, prepared according to the method of Ferry, seem to be nonspecific and of questionable diagnostic significance.

(2) A certain proportion of white mice can be immunized against doses of virulent meningococci by injections of a meningococcus broth filtrate.

(3) The nature of the substances present in meningococcus broth filtrates is probably varied and demands further investigation.

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CLINICAL INVESTIGATION OF INCREASED FRAGILITY OF THE ERYTHROCYTES*

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A DETERMINATION of the resistance of the erythrocytes to hypotonic saline solution has been done as a routine procedure on all blood dyscrasias admitted to the University Hospital. This fragility test has also been done in a large group of miscellaneous diseases as a possible diagnostic aid and in an attempt to compile more complete data concerning these various diseases. Since 1927, 1,750 fragility determinations have been made. This report is a brief analysis of those in which the fragility of the erythrocytes was found to be increased.

The technique which has been employed is that described by Todd and Sanford.¹ A stock solution of 0.5 per cent sodium chloride is carefully prepared and kept tightly stoppered. Uniform drops of this solution are added to a series of 12 tubes, so that the first contains 14 drops and the last one 25 drops. Using the same pipette, distilled water is added until each tube has a total of 25 drops. The dilutions thus obtained range from 0.28 per cent to 0.5 per cent sodium chloride, and by using a 1.0 per cent solution of saline, dilutions above 0.5 per cent can be prepared when desired. Venous blood is obtained in a clean, absolutely dry syringe, and a drop is placed in each tube. The tubes are inverted several times so that the blood and hypotonic saline are thoroughly mixed, and then are allowed to stand for two hours at room temperature. Beginning hemolysis is considered to occur in that tube in which the supernatant fluid is just tinged with hemoglobin, and complete hemolysis in the tube in which the "button" of unhemolyzed sedimented cells has completely disappeared. Other methods for determining the fragility of erythrocytes have been suggested.²⁻⁴ Some of these may be more accurate, but are either too difficult or time consuming for use as a routine procedure.

Hemolysis when determined by this means normally begins at 0.44 per cent saline and is complete at 0.34 per cent. When it begins at 0.48 per cent, it is considered as definitely abnormal. In the 1,750 determinations, 126 patients showed an increased fragility. The clinical records of 111 of these patients were available and constitute the material for this analysis. Table I shows the diagnosis in each of these patients.

Twenty-five (22.5 per cent) of the 111 cases with increased fragility of the erythrocytes occurred in patients with myelogenous leukemia. Eleven (44 per cent) of these had hemolysis beginning at 0.5 per cent sodium chloride or higher, and hemolysis was complete at an average of 0.38 per cent saline, thus showing

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an increase in both beginning and complete hemolysis. During the period covered by the study, 129 cases of myelogenous leucemia were studied in this Hospital, so that 19.3 per cent had an increased fragility. That such a large percentage of the cases of myeloid leucemia would show an increased fragility was quite unexpected. None of these patients presented any clinical manifestations of hemolysis so that excessive hemolysis apparently did not play a part in the production of the anemia in these patients. In a review of the recent literature, two references have been found dealing with changes in the fragility of the erythrocytes in myeloid leucemia. Daland and Worthley⁴ found a decreased minimal resistance and an increased maximal resistance in seven cases of this disease and Watkins, in reporting some atypical blood dyscrasias, stated that he had found hemolytic features in the early stages of myelogenous leucemia.

TABLE I

DIAGNOSIS	NO. OF CASES
Myelogenous leukemia	25
Lymphoma	6
Pernicious anemia	7
Hemolytic icterus (congenital)	18
Hemolytic icterus (acquired)	11
Anemia of pregnancy	8
Hypochromic anemia	8
Myxedema	3
Polycythemia	2
Albers-Schonberg disease	1
Icterus neonatorum gravis	1
Infectious mononucleosis	1
Gaucher's disease	1
Monocytic leukemia	1
Acute leukemia	1
Lobar pneumonia	1
Carcinomatosis of bone marrow	3
Miscellaneous	13
	111

Although an increased fragility was common in leucemic myelosis, it was much less frequent in other types of leucemia. Only 4 cases of lymphatic leucemia were found with an increased fragility, all of which were in the chronic, slowly progressing form in adults. There was also one case of acute leucemia in childhood, and one case of monocytic leucemia showing these changes. Two other cases of lymphoma showed an increased fragility, one being a case of the lymphoblastic type, and one a lymphocytic type without leucemia. The increase in the fragility in this group was not as marked as was found in myelogenous leucemia (Table II). Gaishoch,⁶ in studying a wide variety of diseases, reported one case of lymphatic leucemia which showed no change in fragility, and Daland and Worthley⁴ found no change in three cases.

Seven of the 111 cases had typical pernicious anemia. The hemolysis began on an average slightly higher than 0.48 per cent, and was complete at 0.34 per cent or above. During the period included in this analysis, 748 patients with pernicious anemia have been seen in this clinic, so there was increased fragility in only 0.093 per cent of the cases. Studies of the fragility change in pernicious anemia by different investigators have resulted in many conflicting find-

ings. Hill⁹ found an increased fragility in some instances, and a decrease in others. Daland and Worthley,⁴ after studying 20 cases, found a normal maximal resistance and an increased minimal resistance of the erythrocytes. They also noted a marked decrease in minimal resistance during the period of reticulo-cyte crises. It has been shown by Daland and Zetzel,⁷ and Mermod and Dock,⁸ that the reticuloocytes themselves are not consistently more easily hemolyzed by hypotonic saline than normal cells. According to these workers, changes in the resistance depend more on the size, shape, and hemoglobin content of the cells than on their age.

The fragility of the erythrocytes is characteristically increased in hemolytic icterus; this increase is usually quite marked, but varies from time to time dur-

TABLE II

DISEASE	VARIATIONS IN MIN. RESISTANCE	VARIATIONS IN MAX. RESISTANCE	AVERAGE MIN.	RE- SISTANCE MAX.	NO. CASES
Myelogenous leucemia	0.54 - 0.48	0.46 - 0.32	0.494	0.36	25
Lymphoma	0.50 - 0.48	0.40 - 0.32	0.483	0.375	6
Pernicious anemia	0.50 - 0.48	0.38 - 0.32	0.483	0.360	7
Anemia of pregnancy	0.52 - 0.48	0.40 - 0.32	0.492	0.360	8
Familial or congenital hemolytic ic- terus	0.54 - 0.48	0.44 - 0.32	0.509	0.398	18
Acquired hemolytic icterus	0.967 - 0.48	0.52 - 0.30	0.552	0.391	11
Hypochromic anemia	0.50 - 0.48	0.38 - 0.30	0.482	0.345	8
Carcinomatosis with bone metastasis	0.50 - 0.48	0.36 - 0.34	0.486	0.353	3
Polycythemia	0.54 - 0.48	0.30 - 0.30	0.510	0.300	2
Myxedema	0.48 - 0.48	0.38 - 0.30	0.480	0.366	3
Albers-Schönberg disease	---	---	0.52	0.36	1
Icterus neonatorum gravis	---	---	0.48	0.38	1
Infectious mononucleosis	---	---	0.50	0.38	1
Gaucher's disease	---	---	0.48	0.38	1
Acute leucemia	---	---	0.50	0.32	1
Monocytic leucemia	---	---	0.50	0.38	1
Lobar pneumonia	---	---	0.48	0.36	1
Miscellaneous	0.50 - 0.48	0.38 - 0.30	0.482	0.348	13
					111

ing the course of the disease, and occasionally is not present. In 18 of the 111 cases, the patients had typical manifestations of familial or congenital hemolytic jaundice. Here the variations from normal were quite marked, with both beginning and complete hemolysis occurring well above the normal values. The average figures given in Table II for beginning hemolysis are too low, since in some instances determinations were not carried above 0.5 per cent sodium chloride. Acquired hemolytic icterus, like the congenital or familial type, also shows a rather pronounced increase in the fragility of the erythrocytes, although this change is considered to be less marked and is more frequently absent than in the familial form. A diagnosis of acquired hemolytic icterus was made in 11 of our cases, and in this group the fragility changes were as marked as in the congenital or familial form.

The fragility of the red blood corpuscles is generally found to be either normal or decreased in hypochromic anemia. There were 8 typical cases of hypochromic anemia which had an increased fragility. These 8 cases were about equally divided into the idiopathic variety and that type brought on by chronic hemorrhage. The increase in the fragility was not marked, being above 0.48 per

cent saline in only one case. The diagnosis of anemia of pregnancy was made in 8 cases, and in all of these the anemia was of the hypochromic type. There was no significant reticulocyte increase, no increased van den Bergh reactions, nor any findings suggestive of either a hemolytic icterus or a primary anemia.

The final group is made up of 26 unrelated diseases. In the majority of these, hemolysis began at 0.48 per cent saline and was complete at an average slightly above normal. Three of these had carcinomatosis with bone marrow metastasis, and Waugh¹⁰ has reported 2 similar patients with extensive carcinomatosis of the bone marrow who had a marked hemolytic anemia with an increased fragility. There were 3 patients with myxedema who had a fairly marked secondary anemia and two with polycythemia vera. One case each of Albers-Schönberg disease, Gaucher's disease, infectious mononucleosis, and icterus neonatorum presented a slight increase in the fragility of the erythrocytes. One patient with lobar pneumonia was found with a definite but slight increase in fragility, whereas Needles¹¹ has noted a definite decreased fragility in 30 cases of this disease occurring in the Amazon Valley.

CONCLUSIONS

It is well recognized that the erythrocytes of patients with hemolytic icterus are more easily hemolyzed by hypotonic saline than are those of a normal individual. That there may be similar hemolytic changes in other blood dyscrasias is not so well known. Nearly 20 per cent of the cases of myelogenous leucemia seen in this clinic since 1927 have shown an increased fragility. In addition to the fragility changes several of them had a definite increase in the number of reticulocytes. The combination of increased fragility, reticulocytosis, and an enlarged spleen in an aleucemic myelosis, might certainly lead to a diagnostic error. One of the patients here reported had a leucocyte count of 6,800, an erythrocyte count of 2,300,000, hemoglobin of 39 per cent (Sahli), and a reticulocyte count of 10 per cent. Hemolysis in this case began above 0.5 per cent and was complete at 0.40 per cent sodium chloride. The diagnosis in this case, which was subsequently confirmed at necropsy, was possible only because the blood smear was definitely abnormal and revealed many myelocytes. Other blood dyscrasias may occasionally have an increased fragility at some time in their course, but occur less frequently than in myelogenous leucemia. In a small number of our cases of pernicious anemia, lymphoma, hypochromic anemia, and others, the fragility of the erythrocytes was found to be increased.

Fragility determinations on 1,750 cases were reviewed. Of these, 126 had an increased fragility with hemolysis beginning at 0.48 per cent sodium chloride solution or higher. The records of 111 of these were reported.

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THE EFFECT OF LARGE DOSES OF DIGITALIS ON STANDARD METABOLISM*

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THE determination of the minute volume of the heart in cases of cardiac decompensation has been associated with certain difficulties. As is well known the value of the minute volume (M.V.) is obtained by dividing the standard metabolism by the oxygen absorption per liter of blood, i.e., the arteriovenous oxygen difference. The determination of the latter by means of the method modified by Grollman¹ undoubtedly gives reliable values under normal physiologic conditions, but the results obtained in cases of cardiac decompensation must be regarded with great skepticism. For many years we have often obtained in such cases inconstant results which have neither been due to defective gas analytical technique nor to the patient's not having been able to make the prescribed deep respirations. As an example, a case is here presented where almost daily determinations of the utilization were made for a long period, and where a fully satisfactory technique was employed; but in spite of everything, there was considerable variation in the results obtained (Table I).

It is difficult to say upon what these great variations depend. One might consider an inconstant mixing of alveolar air or possibly variation in blood flow in different parts of the lungs in cardiac decompensation, and especially with pulmonary congestion, something which probably is not met with to any considerable extent under normal physiologic conditions. Sonne and Nielsen,² however, consider that they have observed the first-mentioned even under certain normal physiologic conditions. The great variations in determinations of the minute volume, particularly in cardiac decompensation which are found in the literature, may be explained by the fact that it is difficult to exactly determine the arteriovenous oxygen difference. It is obvious that it would be difficult to arrive at reliable results when it is a question of following the influence of digitalis on the minute volume and stroke volume, respectively, in cases of cardiac decompensation. And, as has been said before, the results given in the

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literature must therefore, in many cases be looked upon as uncertain. Nevertheless, both Grassman and Heitzog¹ and Stewart and Cohn² consider that they have found an increase in the minute volume in these cases. Grassman and Heitzog obtained, however, the same results with both the Grollman method and the Broemser method.

For several years we have, like others,^{3, 7} made the observation that as a rule the standard metabolism is increased in cases of cardiac insufficiency, but as the insufficiency improves a successive decline in the standard metabolism takes place. There is, in certain cases of cardiac insufficiency, a very considerable increase in oxygen consumption during rest and under fasting conditions with relative values of plus 50 per cent and over, values comparable with those met with in hyperthyroidism.

TABLE I

DETERMINATIONS MADE OVER A CONSIDERABLE PERIOD OF THE UTILIZATION OF OXYGEN IN A CASE OF CARDIAC DECOMPENSATION

DATE	ARTERIOVENOUS OXYGEN DIFFERENCE CC O ₂ PER LITER OF BLOOD	MEAN VALUES	GREATEST VARIATIONS
5/26/32	99, 87, 86	91	13 cc
5/27/32	116, 103, 105, 108, 85	103	31 cc
5/28/32	86, 70, 80, 94	84	24 cc
5/30/32	67, 114, 114	98	47 cc
5/31/32	116, 96	106	20 cc
6/ 2/32	94, 82, 60	79	4 cc
6/ 3/32	73, 88, 76	79	13 cc
6/ 4/32	94, 107	101	13 cc
6/ 7/32	81, 72	76	11 cc
6/ 9/32	73, 93, 89	85	20 cc
6/10/32	91, 74, 87	86	22 cc
6/13/32	90, 125, 72, 91, 90, 115	94	53 cc

For the more intensive study of the therapeutic effect of digitalis in the treatment of cardiac decompensation, a series of determinations of the standard metabolism in a number of cases of typical cardiac insufficiency was made, therefore, very soon after the admission of these patients to the clinic, both before and at a definite time after the intravenous injection of large doses of a new preparation of digitalis, "Digiton Leo," which contains both digitalis lanata and purpurea glucosides. The investigations were made under strictly standard conditions, with the patients in a semirecumbent position. Krogh's spirometer was used for determining the standard metabolism, and Recklinghausen's gypotonomograph for determining the blood pressure. The heart rate was determined by means of electrocardiography.

The material comprises 12 cases. The doses of "Digiton" varied from 500 to 1000 frog doses, which would correspond to 0.25 to 0.50 grams of digitalis leaves. In 9 cases there were grave signs of heart insufficiency with dyspnea, anasarca, and considerable enlargement of the liver (Cases 1, 2, 3, 4, 5, 6, 9, 11, and 12). In all these cases extreme enlargement of the heart could be established at the same time. After treatment with "Digiton" and with "Hydrargan Leo" (salyrgan), the edema disappeared in all the cases, with a larger or smaller

decrease in weight. Consequently, the liver was not palpable when the patients were discharged, except in Cases 6, 9, and 11, in whom there was cirrhosis of the liver. Cases 7 and 8 should probably be included in this group representing grave cardiac insufficiency, in spite of the absence of edema and acute swelling of the liver. In the last-mentioned cases, the liver proved to be considerably enlarged but showed no tendency to decrease in size with treatment. Case 10 lacked all signs of cardiac insufficiency during rest and was the only one out of all the cases with a normal-sized heart. In spite of this, he had dyspnea on exertion and showed an obvious insufficiency, with a considerably increased relative oxygen debt in the so-called "stair test." In all the cases with the exception of Cases 9 and 10, the standard metabolism was increased before the injections. As has been mentioned, Case 10 lacked signs of insufficiency during rest. The low standard metabolism of +7 per cent in Case 9 is noteworthy.

In six of the cases (1, 2, 3, 4, 5, and 12) an obvious effect from strong digitalization of the patients was observed only momentarily. Before the injection, the standard metabolism is, as stated before, already increased; but almost immediately after injection it rises to a higher degree. This further increase in the standard metabolism was gradually followed by decreased oxygen consumption, so that in Cases 3, 4, 5, and possibly 12, within an hour after the injections, the standard metabolism lay considerably below the initial value before injection. In five cases, where examinations were made several days in succession, the initial values the day following injection were lower than on the previous day and were followed by a renewed increase within a certain time after the injections. This appears particularly clear in Cases 1, 2, and 12. The mean value of the standard metabolism in these six cases (1, 2, 3, 4, 5, and 12), at the beginning of the investigations exceeded the normal value by 42 per cent, and after the injection of "Digiton" was followed by a mean maximum value of +65 per cent, subsequently with a mean minimum value of +28 per cent. In these six cases of severe cardiac decompensation without cirrhosis of the liver, the maximal momentary increase in oxygen consumption after injection of "Digiton," on the average, amounted to 47 c.c., with a mean error of ± 7 c.c. In these cases it is not only the standard metabolism which is momentarily increased after the injection of "Digiton" but also the systolic blood pressure. (In Case 4 the blood pressure was not determined.) It is striking that at the beginning of the investigation all the cases showed auricular fibrillation with a heart rate of 100 or over. After several daily administrations of "Digiton," there was in all cases a considerable fall in the ventricular rate to 40 to 50 per minute. Also in all the cases already mentioned (1, 2, 3, 4, 5, and 12), the daily treatment with "Digiton" resulted not only in decreasing standard metabolism but also in simultaneous improvements in the patients with considerable diuresis, loss of weight, and disappearance of the swelling of the liver.

In the remaining six cases (6, 7, 8, 9, 10, and 11), the effect of daily administrations of digitalis intravenously on the standard metabolism is more doubtful or entirely absent; but it should be mentioned that these cases were not followed as closely as the former group. Attention should, however, be

called to the remarkable fact that in all of them there was emphysema of the liver, except in Case 10 in which insufficiency during rest was not present. In these cases the liver showed no tendency to decrease in size in spite of intensive digitalis therapy combined with mercury diuretics. Signs of a decrease in heart rate were also absent except in Case 9 where there was some decrease. It is also striking that only this latter case exhibited auricular fibrillation.

DISCUSSION

As in a number of both older and more recent investigations, results are here presented which clearly prove that there is a raised standard metabolism in typical cases of cardiac insufficiency. The mean values for the 11 cases at the first examination amount to a mean of +40 per cent. The explanation of this increased oxygen consumption is disputable. Eppinger⁸ has found an increased lactic acid content in the blood in cardiac insufficiency even during rest, but above all, after work. This latter condition has been verified among others by Jerwell,⁹ Meakins and Long.¹⁰ A deficient resynthesis takes place with an increased oxygen consumption (Hill's "oxygen debt"). It would, therefore, be of the greatest interest to investigate whether or not there is any parallelism between the increased standard metabolism and the lactic acid concentration in the blood, but such an investigation would probably call for the puncture of the right auricle, for it is not certain that the venous blood from the extremities and from the portal system have the same lactic acid concentration. On the contrary, it is possible that it is the amount of congested blood in the liver, caused by cardiac insufficiency in certain cases, which is responsible for the increased oxygen consumption.* The momentary further increase in the standard metabolism, called forth by means of "Digiton," might perhaps be explained in this way. As far as can be judged from this investigation (the material being still small in amount), this momentary increase in the standard metabolism due to treatment with "Digiton" takes place, on the whole, only in the cases where there is acute congestion of the liver, but, on the other hand, not in those cases of insufficiency which are associated with emphysema of the liver. (It is possible that some of the cases labeled emphysema may have been due in some degree to congestion.)

In such recent works as that by Grollman, Møller¹¹ and Weese,¹² it is fairly definitely assumed that the minute volume of the heart is increased by digitalis, which is extremely probable since the standard metabolism and the blood pressure increase. The result is that there is a considerable increase in the stroke volume because the cardiac frequency decreases so appreciably. This latter circumstance should lead to a momentary increase in the volume of the heart also, a matter about which, however, we have no knowledge. As has been mentioned we have observed after injections of "Digiton," at first a momentary increase followed later by a considerable decline in the standard metabolism, a falling blood pressure, probably followed by a decrease in the minute volume stroke volume, and heart volume. For example, in Case 12 a decrease in the size of the heart even up to 400 cc appeared.

*It would have been of interest to determine the standard metabolism under the influence of morphine while the oxygen increasing tendencies of the respiratory muscles were diminished.

CONCLUSIONS

1. In cases of considerable cardiac insufficiency with congestion, swelling of the liver, and anasarca, the standard metabolism has been found to be appreciably increased.

2. In cases without cirrhosis of the liver, "Digiton" in large doses gives rise to a momentary further increase in the standard metabolism and blood pressure, and a reduction in the heart rate, following later by a considerable reduction in the standard metabolism and blood pressure. Associated with these changes there is an improvement in the patient, with reduction in the size of the swollen liver and considerable relief from edema.

3. In cases of cardiac insufficiency with cirrhosis of the liver, digitalization does not seem to cause a momentary increase in the standard metabolism.

CASE REPORTS

CASE 1.—Thirty-nine-year-old male coach painter: Cardiac decompensation with syphilis (mitral insufficiency and myocardial degeneration). Electrocardiogram indicates the possibility of coronary disease.

History.—Patient was a rather keen sportsman and football player. He was perfectly healthy until the middle of April, 1935. Then he began to feel shortness of breath and had palpitation when he walked upstairs. He was unaware of any venereal infection. Previously he had been treated at the clinic for cardiac decompensation in 1935. He again received treatment at the clinic from Dec. 27, 1935, to Feb. 25, 1936.

DATE		STANDARD METABOLISM IN C.C. O ₂ PER MIN.	B.M.R. %	BLOOD PRES- SURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
12/27/35						71	
12/28/35	Before injection	303	+28	125/98	138		400
	23 min. after injection of 500 F.D.* "Digiton Leo"	328	+39	140/82	108		
	50 min. after injection	311	+32	—	108		
12/29/35							2300
12/30/35	Before injection of 500 F.D. "Digiton Leo"	255	+ 8	125/78	108		
	15 min. after	276	+17	135/80	78		3300
	30 min. after	276	+17	135/85	74		
12/31/35	Before injection of 500 F.D. "Digiton Leo"	227	± 0	110/75	72	66	
	15 min. after	253	+12	140/82	72		
	30 min. after	253	+12	140/80	66		
1/ 1/36							2000
1/ 2/36	Before injection of 500 F.D. "Digiton Leo"	253	+12	125/80	84		
	15 min. after	261	+15	125/80	72		1300
	30 min. after	269	+19	120/80	68		
1/ 3/36	Before injection of 500 F.D. "Digiton Leo"	252	+15	115/70	72	63	
	15 min. after	251	+15	118/72	68		
	30 min. after	242	+10	120/72	54		

*Frog doses.

Examination on Admission—General condition fairly good Height 171 cm, body weight 71 kg Temperature (febrile) Sedimentation rate (Westergren), 5 mm per hour Urine 0 Wassermann positive Thyroid not palpable

Examination of the Circulation—Physical Examination Extensive edema of the legs, involving also the undersides of the thighs and pronounced periorbital edema Slight general cyanosis, and slight yellow coloring of the sclerae Moulengracht index of the blood serum 1.15 Liver palpable one hand's breadth below the costal arch Veins of the neck were greatly distended when the upper part of the body was at an angle of 30° Circulation time measured by the decholin method, 48 seconds Blood pressure 110/70 Circumference of the abdomen 92 cm Heart Apex impulse palpable at the fifth interspace just outside the mid clavicular line, diffuse and somewhat heaving Prolonged apical systolic murmur, sounds distant

X ray of the Heart—Heart measured 18.5 cm in length, 14 cm in width, and 12.5 cm in sagittal direction. Volume about 1,360 cc, corresponding to 780 cc per square meter body surface Heart showed enlargement mainly to the left and posteriorly Pronounced pulmonary congestion On the right side there was a slight effusion in the pleura

Electrocardiogram—Rhythm irregular Rate 140 per min QRS interval 0.07 T wave slightly upright in all leads Deflections fairly small and ventricular complexes slightly notched in all leads

Diagnosis—Auricular flutter 2 to 1 3 to 1

Course—Patient was sent to bed and put on 1,000 cc fluid daily The day after admission, Dec 27, the first determination of the standard metabolism was made Liver was not palpable when the patient was discharged

CASE 2—Fifty seven year old male coal shoveler Severe cardiac insufficiency (mitral insufficiency and myocardial degeneration)

Physical Examination on Admission Feb 11 1936—Marked edema with liver enlargement Pulse deficit 28 per min

X ray of the Heart—Pulmonary congestion Heart volume 500 cc per square meter of body surface

Electrocardiogram—Auricular fibrillation

Course—Lost 7.4 kg weight during treatment Liver slightly palpable when discharged

DATE		STANDARD METABO- LISM IN CC O ₂ PEP MIN	B.M.R. %	BLOOD PRES- SURE	HEART PUMP	WT KG	DIAPHRAGM PEP 24 HOURS
2/12/36	Before injection of 1000 FD "Digiton Leo"	281	+49		120	57.3	1000
	15 min after	272	+43		70		
	30 min after	339	+79		60		
2/13/36	Before injection of 1000 FD "Digiton Leo"	196	+4	125/95	80		
	15 min after	213	+13	140/90	50		1100
	30 min after	230	+22	130/85	40		
	35 min after	256	+35				
2/14/36		214	+8			54.6	

CASE 3—Sixty six year old male brewer Severe cardiac insufficiency (mitral insufficiency and myocardial degeneration)

Physical Examination on Admission Feb 24, 1936—Severe edema Liver enlargement

X ray of the Heart—Marked enlargement, 2,000 cc

Electrocardiogram—Auricular fibrillation and partial bundle branch block

Course—Loss of weight up to March 6, 35 kg Liver not palpable at time of discharge

DATE		STANDARD METABO- LISM IN C.C. O ₂ PER MIN.	B.M.R. %	BLOOD PRES- SURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
2/25/36	Before injection of 1000 F.D. "Digiton Leo"	312	+37	160/128	110	82.5	800
	Immediately after	407	+79				
	14 min. after	416	+82		100		
	28 min. after	404	+77	180/90	90		
	48 min. after	321	+41		80		
	60 min. after	377	+65	175/105	80		
	75 min. after	328	+44	180/120			
	100 min. after	268	+18	180/120			
2/26/36	Before injection of 1000 F.D. "Digiton Leo"	303	+33	160/115	90	82.4	2300
	7 min. after	258	+13		60		
	15 min. after	314	+38		58		
	30 min. after	266	+17	180/105			
	40 min. after	253	+13	170/105			
2/27/36	Before injection of 1000 F.D. "Digiton Leo"	244	+9			80.4	2150
	Immediately after	240	+7				
	40 min. after	209	-7				

CASE 4.—Sixty-seven year-old male farmer: Severe cardiac insufficiency (hypertension and myocardial degeneration).

Physical Examination on Admission.—Slight edema, marked enlargement of the liver.

X-ray of the Heart.—Generalized cardiac enlargement; total volume, 1,640 c.c.

Electrocardiogram.—Auricular fibrillation and left axis deviation.

Course.—Lost 5 kg. weight during first two weeks. Liver not palpable at time of discharge.

DATE		STANDARD METABO- LISM IN C.C. O ₂ PER MIN.	B.M.R. %	BLOOD PRES- SURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
3/1/36	Before injection Injection of 1000 F.D. "Digiton Leo"	355	+76	190/115	110	70	
	3 min. after	384	+91	205/110			
	13 min. after	393	+95				
	30 min. after	360	+78	210/105	90		
	42 min. after	317	+57		100		
3/2/36	Before injection of 750 F.D. "Digiton Leo"	317	+57		80	70	500
	Immediately after	301	+49				
	15 min. after	256	+27		60		

CASE 5.—Sixty-six-year old male watchman: Cardiac insufficiency (hypertension, myocardial degeneration, and coronary disease).

Physical Examination on Admission.—Slight edema, marked enlargement of the liver.

X-ray of the Heart.—Marked enlargement of the heart, 2,100 c.c., or 1,100 c.c. per square meter of body surface.

Electrocardiogram.—Auricular fibrillation, bundle-branch block, and ventricular extrasystoles.

Course.—Loss of 1.2 kg. in weight. Liver slightly palpable at time of discharge.

DATE		STANDARD METABO- LISM IN CC O ₂ PER MIN	B.M.R. %	BLOOD PRESS SURF	HEART RATE	WT KG	MURKINS 111 21 HOURS
3/4/36	Resting before injection	328	15		70	78.5	1100
	Immediately after injection of 1000 F.D. "Digiton Leo"	320	11				
	20 min after	345	55		50		
	45 min after	268	21		50		

CASE 6—Forty five year old male barber Cardiac insufficiency with cirrhosis of the liver

Physical Examination on Admission, March 10, 1936—Severe anasarca, ascites, and liver enlargement

X ray of the Heart—Pulmonary congestion, generalized cardiac enlargement, 1,610 cc, or 1,300 cc per square meter of body surface

Electrocardiogram—Normal

Course—Weight loss 25 kg No change in size of the liver

DATE		STANDARD METABO- LISM IN CC O ₂ PER MIN	B.M.R. %	BLOOD PRESS SURF	HEART RATE	WT KG	MURKINS 111 21 HOURS
7/11/36	Before injection of 1000 F.D. "Digiton Leo"	781	+24	128/60	100	107.8	400
	10 min after	356	+16	137/60	90		
	30 min after	309	± 0		90		
	45 min after	226	+ 6	125/65	90		
	90 min after	360	+17				
3/12/36	Resting before injection of 1000 F.D. "Digiton Leo"	467	+52		110	108.0	600
	3 min after	492	+60		100		
	10 min after	188	+38		100		
	30 min after	463	+50		100		

CASE 7—Forty four year old male engineer Cardiac insufficiency with cirrhosis of the liver (mitral and aortic insufficiency, myocardial degeneration, and coronary disease)

Physical Examination on Admission—No edema, enlargement of liver, pulmonary congestion

X ray of the Heart—Pulmonary congestion, generalized cardiac enlargement, 1,500 cc (850 cc per square meter of body surface)

Electrocardiogram—Partial auriculoventricular block, bundle branch block, ventricular extrasystole

Course—No change in size of the liver Loss of weight 2 kg at time of discharge

DATE		STANDARD METABO- LISM IN CC O ₂ PER MIN	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT KG
4/9/36	Before injection of 750 F.D. "Digiton Leo"	312	44%	155/70	84	60.5
	8 min after	311	+62	169/85	84	
	20 min after	328	+55	170/90	84	
	40 min after	320	+52	155/85	84	

CASE 8.—Seventy-two year old male electrician: Cardiac insufficiency with cirrhosis of the liver (mitral and aortic insufficiency; myocardial degeneration, and coronary disease).

Physical Examination on Admission.—No edema, moderate enlargement of the liver.

X-ray of the Heart.—Severe pulmonary congestion, generalized cardiac enlargement, 2,100 c.c. (1,250 c.c. per square meter of body surface).

Electrocardiogram.—Partial atriocentric block and bundle branch block.

Course.—Weight loss 9 kg. No change in size of liver at the time of discharge.

DATE		STANDARD METABO- LISM IN C.C. O ₂ PER MIN.	B M R. %	BLOOD PRES- SURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
4/14/36	Resting before injection of 875 F.D. "Digiton Leo"	324	+73	148/53			63
	10 min. after	315	+69	158/53			
	21 min. after	306	+64				
	44 min. after	352	+88	145/48			

CASE 9.—Seventy year old male cobbler: Cardiac insufficiency with cirrhosis of the liver.

Physical Examination on Admission.—Slight edema, ascites, marked liver enlargement.

X-ray of the Heart.—Generalized cardiac enlargement, 1,800 c.c. (1,050 c.c. per square meter of body surface).

Electrocardiogram.—Auricular fibrillation, partial bundle branch block.

Course.—No diminution in size of the liver. Loss of 7.4 kg. weight.

DATE		STANDARD METABO- LISM IN C.C. O ₂ PER MIN.	B M R. %	BLOOD PRES- SURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
3/16/36	Before injection of 750 F.D. "Digiton Leo"	211	+ 7	145/ 95	60	68.4	600
	10 min. after	207	+ 5	150/102	40		
	30 min. after	164	-17	145/ 95	50		

CASE 10.—Forty nine year old male carpenter: Dyspnea on exertion.

Physical Examination on Admission.—No edema, no liver enlargement.

X-ray of the Heart.—Normal heart volume, 450 c.c. per square meter of body surface.

Electrocardiogram.—Left axis deviation.

Heart Function Tests.—Nylin's "Stan Test" showed marked insufficiency with slight test.

Course.—Loss of body weight, 3.05 kg.

DATE		STANDARD METABO- LISM IN C.C. O ₂ PER MIN.	B M R. %	BLOOD PRESSURE	HEART RATE	WT. KG.
4/21/36	Before injection of 750 F.D. "Digiton Leo"	286	+14	120/75	72	85
	10 min. after	257	+ 2	118/78	66	
	22 min. after	252	± 0	110/72	72	
	40 min. after	278	+11	120/82	72	

CASE 11.—Fifty seven year old male carpenter. Marked cardiac insufficiency with cirrhosis of liver (mitral insufficiency, and coronary disease).

Physical Examination on Admission.—Marked edema of the legs, marked enlargement of the liver.

X ray of the Heart—Generalized cardiac enlargement, heart volume 1,480 cc (900 cc per square meter of body surface)

Electrocardiogram—Bundle branch block

Course—Loss of weight 7 kg No change in size of liver at time of discharge

DATE		STANDARD METABO- LISM IN CC O ₂ PER MIN	B M P %	BLOOD PRESSURE	HEART RATE	WT KG
7/21/36	Before injection of 1000 FD "Digiton Leo"	248	+34	120/90		
	10 min after	242	+31			
	20 min after	250	+35	130/95		
	40 min after	267	+44	140/95		
7/22/36	Before injection of 1000 FD "Digiton Leo"	264	+43	120/80	84	
	10 min after	267	+44	128/90	78	
	20 min after	276	+49	120/80	72	
	40 min after	267	+44	123/85	72	

CASE 12—Forty one year old trolley car operator Severe cardiac insufficiency (mitral insufficiency and myocardial degeneration)

History—History of acute polyarthritis in 1911 but following this he was healthy and worked hard Dyspnea since Feb, 1936 Edema of the legs since three weeks before admission

DATE		STANDARD METABO- LISM IN CC O ₂ PER MIN	B M P %	BLOOD PRES- SURE	HEART RATE	WT KG	DILPESIS PFT 24 HOURS
5/26/36	Before injection of 1000 FD "Digiton Leo"	282	+18	120/100	80	75.5	
	25 min after	321	+37	145/100	90		
	40 min after	273	+14	140/98	90		500
5/27/36	Before injection of 750 FD "Digiton Leo"	230	- 4	135/ 80	70		
	25 min after	277	+15	140/ 85	90		
	40 min after	267	+11	145/ 80	100		1100
5/27/36					36		
5/28/36	Salysrgan (2 cc intraven- ous)						5600
5/29/36	Before injection of 500 FD "Digiton Leo"	212	- 5	148/ 80	60		
	20 min after	234	+ 5	150/ 88	60		
	40 min after	243	+ 9.5	148/ 80	70		
	60 min after	243	+ 9.5	165/110	60	69.4	1700

Physical Examination on Admission May 25 1936—General condition bad No temperature Sedimentation rate (Westgren) normal Weight 75.5 kg Thyroid not enlarged Urine albumin ++ Marked edema over nearly the entire body Ascites Liver markedly enlarged Blood pressure 140/75

Heart Apex impulse felt in fifth interspace outside the midclavicular line. No apical systolic murmur Apical first sound accentuated, also second pulmonic sound No pulse deficit

X-ray of the Heart.—Pulsations of the heart were small. Length of heart, 23 cm.; breadth, 19 cm.; saggital diameter, 16 cm.; generalized enlargement of the heart with bulging especially over region of left auricle. Pulmonary congestion.

Electrocardiogram.—Auricular fibrillation, partial bundle-branch block.

Course.—Weight loss, 20 kg. No liver enlargement at time of discharge.

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NONHEMOLYTIC STREPTOCOCCIC MENINGITIS: REPORT OF A CASE SUCCESSFULLY TREATED WITH SULFANILAMIDE AND PRONTOSIL

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SINCE the initial work by Domagk¹ on the chemotherapy of streptococic infections with sulfanilamide, numerous other workers in this field have reported cases successfully treated with this drug, although no case of nonhemolytic streptococic meningitis has heretofore been recorded. Among the significant papers are those of H. J. Gray,² who reviewed the literature on streptococic meningitis up to July, 1935; Caussé, Loiseau, and Gisselbrecht,³ who reported a case of hemolytic streptococic meningitis with recovery after the use of prontosil; Buttle, Gray, and Stephenson;⁴ Long and Bliss,⁵ whose paper contains a notable bibliography on para-amino-benzene-sulfonamide and its chemical derivatives; Proom,⁶ and Anderson,⁷ who likewise reported a case of hemolytic streptococic meningitis which recovered rapidly when prontosil was given by mouth and intramuscularly; Mellon, Gross, and Cooper,⁸ who reported on their experimental studies with sulfanilamide in mice; and Weinberg, Mellon, and Shinn,⁹ who reported two cases of meningitis, both of hemolytic streptococic, and both recovered on sulfanilamide and prontosil.

Of particular interest is the statement by Anderson:⁷ "The consensus of all the authors seems to be that prontosil is of value in the treatment of hemolytic streptococic infection, but it apparently has little or no effect on other strains of streptococci or other organisms."

Reference should also be made to the Report of the Council on Pharmacy and Chemistry on Sulfanilamide and Related Compounds,¹⁰ May 29, 1937, and particularly to the statement, "No success has been reported for the treatment of infections due to alpha and non hemolytic streptococci" with sulfanilamide

The purpose of this paper is to report a case of nonhemolytic streptococcal meningitis of marked severity which has been successfully treated by the use of sulfanilamide and prontosil. The organism was gamma nonhemolytic streptococcus*. It produced no hemolysis in aerobic or anaerobic culture. It was gram negative. Successive cultures of the organism grew well on blood agar, but no hemolysis occurred. Culture plates of *Streptococcus viridans* and hemolytic streptococci, for comparison, showed typical growths.

This case is of further interest because of the large amount of sulfanilamide which was given. Toxic symptoms resulted from this large dosage, but recovery took place promptly and without ill effects. Effort has been made to

TABLE I

DATE	SMEAR	CULTURE	CELL COUNT	SP FL SUGAR	SULFANILAMIDE IN SP FL (PER CC)
5/25/37	Positive	Positive	1,100		
5/26	Positive	Positive	2,660		
5/27	Positive	Positive	3,360		
5/28	Positive	Positive	3,680	5.4 mg	
5/29	Positive	Positive	3,995	3.0 mg	
5/30	Positive	Positive	2,220	6.1 mg	
5/31	Positive	Positive	2,905	15.6 mg	
6/ 1	Positive	Positive	3,550	10.9 mg	Positive
6/ 2	Positive	Positive	3,120	7.8 mg	Positive
6/ 3	Negative	Negative	1,472	12.8 mg	Positive
6/ 4	Positive	Positive	908	14.7 mg	0.35 mg
	one chain				
6/ 5	Negative	Negative	881	18.6 mg	0.3 mg
6/ 6	Negative	Negative	292	23.2 mg	0.125 mg
6/ 8	Negative	Negative	775	18.3 mg	
6/10	Negative	Negative	502	21.7 mg	
6/14	Negative	Negative	93	18.4 mg	

In this table there is no correlation between the spinal fluid sugars and the blood sugar inasmuch as it was not feasible to take blood sugars at this time.

correlate the clinical and chemotherapeutic findings by qualitative and quantitative estimations of the amount of the drug in the spinal fluid, together with estimations of the spinal fluid sugar, cell counts, etc. (Table I).

The total quantity of sulfanilamide given by all routes was 1,164 grams. In this case the most effective method of introducing it was by rectum, because much of that given by mouth was promptly vomited (not included in total dosage), and there seems to be no good reason for introducing it intravenously. Intramuscular injections of 25 per cent prontosil were started when the diagnosis was made, and this was followed by oral and rectal administration. None was administered directly into the cistern or the spinal canal.

In addition to the drug therapy, cisternal and lumbar punctures were done as seemed advisable, the drainage each time being sufficient to lower the excessive pressure. Blood transfusions were given on four occasions. Glucose in

*I am indebted to Dr. R. F. Sledge, Lieut. Com. M. C. U. S. Navy, for the bacteriologic work in this case.

saline was given as necessary to combat carbohydrate deficiency, acidosis, and water loss. It is, however, the distinct clinical impression that these were but secondary considerations in the recovery and that most credit for the successful outcome was due to the para-amino-benzene-sulfonamide. When the drug was given by rectum, 30 gr. of the powder was dissolved in 2 oz. of warm saline solution, thereby increasing the concentration the patient received from 2.5 per cent to approximately 4 per cent.

CASE REPORT

History.—J. C., a white female, aged six and one-half years, began to complain of an earache in the right ear on May 7, 1937, and the next day was taken to the Naval Hospital Dispensary where myringotomy of the right eardrum was done by Dr. J. H. Hooker. The ear drained copiously. Unfortunately, no smear or culture is available of the initial pus.



Fig. 1.—Streptococci in spinal fluid smear, May 31, 1937.

The patient is the youngest of four living children. The mother and father are living and well. Her past history was entirely negative, except for chickenpox in 1931, pertussis and German measles in 1934. No sequelae of either had been noted. She had never had any ear trouble prior to this attack.

Four days after the onset (May 11), x-ray of the mastoid was reported: "The films are not very clear, but the cells on the right side, superiorly, appear slightly blurred." From May 10 to May 20, she received a small series of x-ray treatments: to the right ear area, 282 r. units in three days, 200 r. units to the right mastoid area on the next two days, and 70 r. units to the pharynx on the fifth day. This treatment was apparently without beneficial effect.

She was first seen by the author on May 22, because it was thought that she was developing measles which was an epidemic in the community at the time.

Examination.—The child was well developed and nourished, mentally clear, alert, quiet, and cooperative. There was moderate coryza and a slight maculopapular rash on the neck and upper extremities and Koplik spots on the nasopharyngeal mucosa. There was purulent drainage from the right ear canal, and the site of the myringotomy was patent. The temperature by mouth was 104°. There was no evidence of meningeal irritation, mastoid tender-

ness or smoothness, headache, and she appeared not acutely ill. The examination was otherwise not remarkable. No reflex changes were noted at the initial examination.

Subsequent Course—Forty-eight hours later, however, she had developed evidence of meningitis. The lids drooped bilaterally, her temperature had risen to 106.8° by rectum, and there was marked irritability, tonic, and slight stiffness of the neck without rigidity. The eruption of measles was widespread. The knee jerks were hyperactive, Kernig's and Babinski's signs were positive on both sides, and an inextinguishable ankle and patella clonus was elicited. She complained of mild headache.

She was admitted to Alexandria Hospital at midnight on May 25. At this time she was slightly irrational, disoriented, and beginning to grow stuporous. The neck had become progressively more rigid. Lumbar puncture was performed immediately on admission. There was some increase in the pressure, and the fluid was faintly opalescent. At this time 10 cc of antimeningococcal serum was given intraspinally, and the fluid was sent to the laboratory for study.

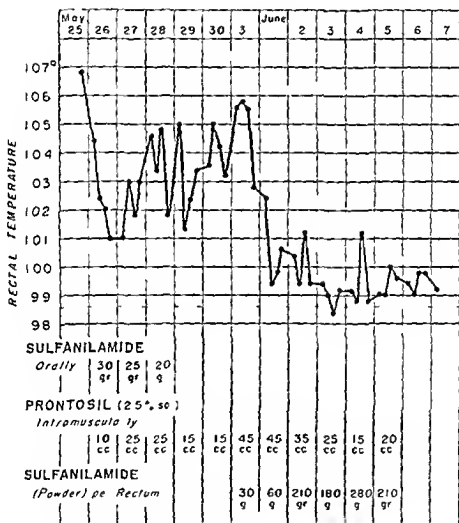


Fig. 2—Temperature curve and doses of sulfanilamide in case of nonhemolytic streptococcal meningitis.

Examination of the smear showed numerous short and long chains of gram-negative streptococci (Fig. 1). As soon as the diagnosis of streptococcal meningitis was made, prontosil was begun intramuscularly and sulfanilamide tablets were given by mouth (Table II). Because of vomiting, however, the method of administration was changed after three days to a solution of powder by rectum. None of this was expelled. The qualitative test of the spinal fluid for sulfanilamide was first reported as positive on June 1, and on June 4, the concentration in the spinal fluid had reached 0.35 mg per cubic centimeter of fluid. The following day this concentration was reported to be 0.3 mg per cubic centimeter and decreased to 0.125 mg two days later, in spite of the large dosage given.*

*Mr. P. T. Rees, Chief Pharmacist U. S. Navy, did both the qualitative and quantitative analyses on the spinal fluid.

On May 26, the patient was seen in consultation with Dr. G. Haven Mankin for an opinion on the ear condition and the mastoiditis. His report: "The patient has a small amount of mucopurulent drainage from the right ear and definite tenderness over the right mastoid antrum, marked stiffness of the neck, positive Kernig. Glands at angle of jaw enlarged, throat red, veins in fundi full. Advise treatment of meningitis without operative interference R. mastoid. Suggest hot packs and irrigations R. ear every two hours."

Laboratory Data.—On admission to the hospital the leucocyte count was 19,700 with 86 per cent neutrophils, 12 per cent lymphocytes, 2 per cent large monocytes—juvenile forms 4 per cent—stab 40 per cent. Urinalyses were entirely negative. Blood culture taken two days after admission never showed any growth. The R.B.C. on admission 4,100,000, and hemoglobin 78 per cent (Dare). The patient's blood was Type 2. The stained spinal fluid smear showed innumerable chains of streptococci (Fig. 1). The day following admission the W.B.C. were 16,650 with 92 per cent neutrophils.

TABLE II

DATE	SULFANILAMIDE	PRONTOSIL INTRAMUSCULARLY	SULFANILAMIDE, POWDERED RECTALLY
5/26	30 gr. P.O.	10 c.c.	
5/27	25 gr. P.O.	25 c.c.	
5/28	20 gr. P.O.	25 c.c.	
5/29	None (vomiting)	15 c.c.	
5/30	None (vomiting)	15 c.c.	
5/31	None (vomiting)	45 c.c.	
	Total 75 gr.		
5/31		40 c.c.	30 gr.
6/ 1		45 c.c.	60 gr.
6/ 2		35 c.c.	210 gr.
6/ 3		25 c.c.	180 gr.
6/ 4		15 c.c.	280 gr.
6/ 5		20 c.c.	210 gr.
	Sulfanilamide treatment stopped.		Total 970 gr.
Total dosage 1,164 gr.			

Progress in Hospital.—Daily cisternal and lumbar punctures were done throughout her stay of three weeks in the hospital. Approximately 20 to 40 c.c. of cloudy fluid were removed at each tap. Four small transfusions were given as supportive treatment as follows: on May 26, 200 c.c. of blood, May 28, 200 c.c., May 31, 250 c.c. and on June 2, 250 c.c. By May 31, the eruption of measles had disappeared.

On June 1, the condition of the patient was most critical. Despite withdrawal of spinal fluid and adequate administration of fluids by vein, the situation became desperate. The streptococci were present in increased number in the smears. The cell count was increasing, the culture remaining positive, and the spinal fluid sugar decreasing. The prognosis seemed extremely grave. It was decided, therefore, to introduce a large amount of sulfanilamide by rectum since the patient was not retaining any by mouth. In divided dosage 30 gr. were given dissolved in 2 oz. warm physiologic saline solution. This was increased to 60 gr. in the next twenty-four hours, to 210 gr. the next day, to 180 gr. on the fourth, and to 280 gr. on the fifth day. Thereafter only one further dose was given; 210 gr. on the sixth day of the rectally administered drug (Table II). On the fourth day of this treatment, the smear and culture were negative but were reported positive again on the fifth day. The sixth day and thereafter the spinal fluid smear showed no streptococci, and the culture was reported as showing no growth. None of the drug was expelled from the rectum, and the ease of administration and rapidity of solution attracts itself as a splendid method of giving the drug, especially to children and to patients who are vomiting. Clinical improvement was noticed after the fifth day of the rectal administration of sulfanilamide.

Development of Toxic Symptoms.—These were noticed fairly early during the drug therapy, the earliest and most striking was the now familiar dusky violaceous cyanosis, which was especially noticeable in the lips, finger and toe tips, helices of the ears, etc. As was to

be expected when the dosage was increased, other toxic symptoms became apparent—increased irritability and uneasiness, rather marked weakness, and increased respiratory and cardiac rates. It seems now quite apparent that this overdosage caused most of the latter, because the toxic symptoms quickly subsided when the drug was withdrawn. Although sulfanilamide is apparently quickly excreted by the renal route, it is assumed that with any kidney damage this would not take place, and the resultant cumulative action of this toxic medicament might cause depression and failure of respiratory and cardiac centers. In spite of the enormous dosage the patient rallied rapidly, and, except for continuation of a transient weakness several days after withdrawal, no harmful effects were noted. When streptococci finally disappeared from the spinal fluid, her improvement was rapid, and three weeks after admission to the hospital the patient was discharged to her home.

Blood Examination on the Day of Discharge from Hospital—Hemoglobin, 10 gm, or 87 per cent (H H), RBC, 3,920,000, WBC, 5,350. Differential polymorphonuclears, 76 per cent, lymphocytes, 21 per cent, large monocytes, 3 per cent, RBC showed slight anisocytosis. It is apparent that the drug acts directly on the invading organism. It did not cause leucocytosis and apparently did not severely depress the bone marrow.

CONCLUSIONS

A case of nonhemolytic streptococcic meningitis is reported. Treatment included prontosil and sulfanilamide in large doses, aggregating 1,164 gr. Some symptoms of toxicity are reported. Recovery was uneventful and without sequelae.

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A CONSIDERATION OF QUANTITATIVE RELATIONS BETWEEN ERYTHROCYTES, LEUCOCYTES, AND HEMOGLOBIN OF THE BLOOD*

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ALTHOUGH the origin and function of the red blood cells, the white blood cells, and the hemoglobin of the blood are quite different, each constituent gives an index of the status of the tissues from which it is derived, and in case of pathologic disturbance within the organism, one or all three may reflect alterations in the fluid matrix and other tissues of the body. In the development of these important components of the blood from their original source, there are within the body certain determining, reacting, and participating factors to which all are subjected, and in addition they are exposed to the same environmental influences within the blood stream. It would seem, therefore, that there might exist some quantitative relationships of significance among the three, since they are present in one and the same circulating tissue medium and are intimately intermingled and associated together. Yet the literature seems to contain no record of a mathematical study having been made of the blood of large numbers of normal individuals. Pursuant to a study of the complete blood picture and of the structural and functional changes characteristic of infancy,¹ as well as during later growth and development, an investigation as to the relative behavior of some of the measurements of these blood constituents has been made.

The data discussed herein were selected from some growth studies conducted in the Research Laboratory of the Children's Fund of Michigan, where monthly blood observations were made as a part of the detailed medical examination given a large group of healthy infants during their first year of life, the details of which can be found elsewhere.²⁻⁶ There were 1,644 instances in which simultaneous observations were made of erythrocytes, leucocytes, and hemoglobin at the same clinic visit.

The methods for determining the number of erythrocytes, leucocytes, and the amount of hemoglobin in the blood were carefully standardized, and the bloods were taken at the same time of day, between the hours of 10:00 A.M. and noon. A free blood flow was established from the large toe of each subject by a Bard-Parker knife, number 11. The same Trenner hemoglobin and red blood cell pipettes, certified by the United States Bureau of Standards, were used throughout the study. The blood for the hemoglobin determination was made up

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to a 1:10 dilution in the 10 c mm diluting pipette with $n/10$ hydrochloric acid. The pipette was shaken immediately for two or three seconds and allowed to stand thirty minutes at room temperature for the color reaction to take place in the pipette bulb. The hemoglobin was then determined in the Haden-Hausser hemoglobinometer⁷ which was checked at regular intervals against the Van Slyke gasometric method for estimating hemoglobin.⁸ All observations were made by only three laboratory workers (B. M., M. L. S., and L. E.) to reduce the personal error factor.

Hayem's diluting solution was used for making the erythrocyte counts. A mechanical rotary angular shaker was used for agitating the hemacytometer pipettes,⁶ and after mixing for ten minutes a standard distribution of cells was obtained. Only one Levy-Hausser counting chamber with the improved Neubauer ruling $\frac{1}{400}$ sq mm $\frac{1}{10}$ mm deep, which had been certified by the United States Bureau of Standards, was used in this study. Especial care was taken to keep the counting chamber scrupulously clean, to discard three or four drops from the blood pipette before filling the counting chamber, to fill the chamber as carefully and quickly as possible, and to allow two or three minutes for the settling of the cells before making the red cell count. All the spaces beneath the five double rulings were counted, thus making 5 sq mm in all. The sum of the erythrocytes in these squares was multiplied by 10,000 to give the number of cells per cubic millimeter.

The white cell pipettes were also of the Trenner automatic type and certified by the United States Bureau of Standards. Two per cent acetic acid was used as the diluent, and the pipettes were agitated in the shaker. The five large squares of the counting chamber, including the center, were counted, making eighty small squares in all, and the sum of the cells in these squares was multiplied by forty to give the number of cells per cubic millimeter.

Since the three constituents were not always determined at each presentation of the child for blood samples, the data thus selected present an interesting problem—one entirely incidental to this investigation—in the reliability of results obtained from a small random sample of data as compared to that of the much larger number of measurements from which the sample is chosen. This is shown by the fact that the 3,541 measurements originally studied showed an average of 11.34 gm of hemoglobin per 100 cc of blood, with a standard deviation of 1.25, whereas the sample of 1,644 determinations selected for this study averaged 11.40 gm of hemoglobin per 100 cc of blood, with a standard deviation of 1.38. That the results for this smaller sample are well within the limits of expected variation is obvious without application of further numerical proof.

The data from 1,644 simultaneous determinations of hemoglobin, erythrocytes, and leucocytes on 364 well infants are presented in three so called correlation tables, erythrocyte hemoglobin, hemoglobin leucocyte, and erythrocyte leucocyte, Tables I, II, and III, respectively. In case of the hemoglobin, the frequency classes are designated by the midvalue of the class interval, stated in grams per 100 cc of blood, the frequency classes of the red blood cells are designated by the lower limit of the group, stated in units of 10,000 per c mm, and the white blood cells are measured in units of 100 per c mm, with the lower limit of the class interval being given. It should be noted that the length of the class

T_AHEMOGLOBIN-ERYTHROC
(1644 Blo

ERYTHROCYTES IN UN																							
HEMOGLOBIN IN GRAMS PER 100 C.C. OF BLOOD	178	240	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430			
6.5	1	1																					
7.0		1																					
7.5		1																		1			
8.0									1	1		1	1	2									
8.5						1			1										1				
9.0						1		1	2	2	1	2	1		1	1	2	1	2	1			
9.5		1		1		1	2	2	1	2	2	1	2	2	3	3	1	3	5	7			
10.0		1	1	1	3		1	3	5	9	9	14	13	10	15	11	8	20	10	12			
11.0					1	1	1	6	12	13	12	9	19	28	22	32	35	32	43	44			
12.0					1	1			4	3	8	10	11	9	18	21	25	26	34	36			
13.0								2	1	1	1	5	4	2	3	3	12	16	13	11			
14.0															1	3	3	4	5	2			
15.0													1					1		2			
16.0																			1	1			
17.0																							
18.0																							
\bar{fx}	1	5	1	2	5	5	4	14	27	31	33	43	51	54	65	74	88	104	113	114			
	Hemoglobin = y					Erythrocyte = x					$r_{xy} = 0.26$												
	$M_y = 11.40$					$M_x = 440.20$					$FE_{rx} = 0.02$												
	$\sigma_y = 1.38$					$\sigma_x = 63.34$					$\sigma_c = \sigma_y \sqrt{1-r_{xy}^2} = 1.33$												
											$\frac{\sigma_y}{\sigma_x} \cdot r_{xy} = 0.01$												

T.

HEMOGLOBIN-LEUCOC
(1644 BU

		LEUCOCYTES IN U.																												
		23	40	46	52	58	64	70	76	82	88	94	100	106	112	118	124													
HEMOGLOBIN IN GRAMS PER 100 C.C. OF BLOOD	6.5															1														
	7.0															1				1										
	7.5															1														
	8.0															2	1	1	1	5										
	8.5															5	2			1										
	9.0															2	1	5	2	3	3	2	2	2						
	9.5															1	3	2	6	5	9	5	3	9	3	4	4	4	3	
	10.0															1	2	3	9	11	13	14	24	20	15	18	16	18	21	2
	11.0															1	4	7	16	23	33	46	52	42	52	54	41	39	45	29
	12.0																4	8	13	13	25	30	40	33	43	44	39	36	34	24
	13.0															2	1	1	4	4	8	7	16	14	7	16	11	10	15	10
	14.0																1	1	1	4	2	4	6	3	3	6	5	6	3	2
	15.0																1	1	1			1	1	2	1	3	2	2	1	
	16.0																			1			2	2	1	1	1			
	17.0																	1	1							1		1		
18.0																					1					1				
	f_z	1	3	13	24	49	64	88	123	151	123	138	147	129	117	128	71													
Hemoglobin=y		Leucocyte=z																												
$M_y=11.40$		$M_z=104.20$																												
$\sigma_y=1.38$		$\sigma_z=29.40$																												
		$r_{yz}=0.07$																												
		$r_{r_y z}=0.02$																												
		$\sigma_e = \sigma_y \sqrt{1-r^2_{yz}} = 1.38$																												
		$\frac{\sigma_y}{\sigma_z} \cdot r_{yz} = 0.00$																												

1

DETERMINATIONS OF 364 INFANTS
(DETERMINATIONS)

OF 10,000 PER CMM

450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	670	680	fy
																						2
								1														2
		1						1								1						6
				3					1	1	1											12
				3	1					1						1						10
1		3	1				1	1														25
4	4	2	5	5	3		0	3		1												73
7	15	14	8	5	3	4	4	1	2	1	3	1	1	1								226
34	36	29	25	23	19	21	11	4	10	2	4	2	3				1					568
38	35	25	20	32	17	18	11	5	7	4	2	3	1	4	1							470
9	7	10	10	10	3	2	2	4	2	1	2	3		1	1	1			1	2	151	
1	2	4	5	5	2	1	3	3	1	2		1		2					1		55	
1	1	1	1	3	2	2			2	2	1	1									23	
1					1		2	1	1	1	1	1									13	
	2																			1	5	
							1			1							1	1			3	
96	102	89	75	90	52	49	43	22	27	17	10	12	5	8	4	2	1	1	2	1	2	1044

II

DETERMINATIONS OF 364 INFANTS
(DETERMINATIONS)

OF 100 PER CMM

136	142	148	154	160	166	172	178	184	190	196	202	208	220	226	268	298	fy
																	2
																	2
																	6
																	12
								1									10
		1	2														25
	3	2		1			1			1							73
	7	5	7	4	1	1	3	1		1			1				226
	9	14	6	7	5	7	4	2	1	1	1	1	1	1	1	1	568
	16	7	13	3	10	4	2	3		1	4		1	2			470
	2	5	2	2	1	1	5				1						151
	2	2	1														55
	2				1	3	1										23
	1	1					1										13
							1										5
																	3
42	38	31	18	20	17	16	6	1	2	7	2	2	1	3	1	1	1644

TABLE
LEUCOCYTE-ERYTHROCYTE

		ERYTHROCYTES IN UNITS																						
		178	240	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440		
LEUCOCYTES IN UNITS OF 100 PER C.M.M.	23																							
	40																					1		
	46											1				1	1	3					2	
	52								1			1		2	1	1		2	5	1	3			
	58				1		1	2	1	1			3	2	1	2	4	3	2	2	2	5		
	64			1				1	2	4				3	5		3	1	9	1	5	3	7	
	70		1						2	2	3				6	3	5	5	2	6	4	8	10	
	76				1	1	1	1			2	7	6	2	5	8	4	6	3	12	11	4		
	82		1				1		1	4	1			5	3	5	8	10	5	11	12	13	10	
	88		1				1		2	2	6	3	2	4	4	6	1	9	6	8	7	5		
	94					2	1	1	2	7	3	5	4	4	7	6	5	6	6	12	10	4		
	100								1	2	2	2	3	3	6	3	10	10	9	5	12	7		
	106					1				2	4	1	3	4	7	5	7	8	8	7	6	7		
	112			1	1			1	1			2	1	2	3	3	7	7	7	8	10	8	4	
	118		1						1	3	1	4	4	3	3	2	5	5	14	8	10	9		
	124									1	2	2	1	1	1	5	3	7	5	3	5			
	130			1						1		2	1	2	2		3	2	9	7	5	4		
	136										2		1	1	1	1			4	5		4		
	142							1				1	1	2	1	1	3	1	1	3	3	3		
	148												1	2			4	1	2			2	3	
	154							1								1		1				1	2	
	160														1				2		2	2		
	166													2		1		1	1		1	1	1	
	172									1	1					1	1		1	3	1		1	
	178															1			1		1	1		
	184																							
	190																					1		
	196														1					1			1	
	202																							
	208																				1			
	220																							
	226																						1	
	268																							
	298																							
fx		1	5	1	2	5	5	4	14	27	31	33	43	51	54	65	74	88	104	113	114	97		
		Erythrocyte = x					Leucocyte = z					$r_{xz} = 0.13$												
		$M_x = 440.20$					$M_z = 104.20$					$PD_{rxz} = 0.02$												
		$\sigma_x = 63.34$					$\sigma_z = 29.40$					$\sigma_z = \sigma_x \sqrt{1 - r^2_{xz}} = 29.14$												
												$\frac{\sigma_z}{\sigma_x} \cdot r_{xz} = 0.06$												

interval in the hemoglobin classification is not uniform throughout, the length in the lower half of the table being twice that of the upper half. If we designate the measure of the red blood cells by the letter x, the measure of the hemoglobin by the letter y, and the measure of the white blood cells by the letter z, then the pictures presented by the tables tell us that there are evidently no clearly defined functional relationships of the form $y = f(x)$, or $y = F(x, z)$. The widely used measure of linear relationship between two variable quantities, namely, the Pearson correlation coefficient, is evidently small though from the graphical standpoint, a linear relation in each of the three cases seems to be the most

TERMINATIONS OF 364 INFANTS

0,000 PER CMM																	f ₂
460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	
							1										1
							1		1								3
2			1			1											13
		2	1				1		1								24
3	2	1	2	1	2	3			1								49
5	2	3	1	2	2	1				1							64
4	6	4	2	2	2	3	1	1	1				1			1	86
8	5	4	14	2	2	2			1	1							123
12	15	5	3	3	4	1			2	1			1	1			151
8	4	9	4	2	2	6		2		1	3			1			123
3	5	6	10	3	4	3	2	6	1	1					1		138
13	11	9	8	2	2	6	5		2	2	1		3				147
8	6	6	9	5	4		3	3	3	1	2						2 129
6	5	5	5	4	7	2	2	6		2		1	1				117
7	10	6	13	4	3	3	1		1	1		1				1	128
4		5	6	6	1	2	2	1	1		1				1		71
2	4	2	4	4	3	1			1	2		1		1		1	68
3	3			3	1	4	1		1	1	1					1	42
	3	2	2	3	2	1		1					1	2			88
5	4		1		1	1			1				1				31
1		1		1	1		1	1	1	1	2						18
3	1	1	1	1		1	1				1						20
2	1		1	2	2	1											17
		2			1			1				1					15
					2												6
							1										1
			1														2
		1			1												7
			1			1											2
1							1										2
1																	1
	1										1						3
1																	1
			1														1
102	89	75	90	52	49	43	22	27	17	13	12	5	8	4	2	1	1,644

logical one to assume. For the data of each of the tables the following constants have consequently been calculated for the two variables, say α , β , of the respective tables: (a) the correlation coefficient, $r_{\alpha\beta}$, (b) the probable error of the correlation coefficient, $P.E. r_{\alpha\beta}$, (c) the slope, $m = \frac{\sigma_{\alpha}}{\sigma_{\beta}} r_{\alpha\beta}$, of the line of best fit to the data by the method of least squares (remembering that this line always passes through the means of the two distributions), and (d), the standard error of prediction, σ_p , of values of α from β by this line of best fit.

Of the three constituents under consideration, probably the most often associated in disension are the red blood cells and the hemoglobin. From Table I, which represents the data of these measurements, we find $r_{xy} = 0.26$, $P.E. r_{xy} = 0.02$, $\frac{\sigma_y}{\sigma_x} r_{xy} = 0.01$, and $\sigma_e = 1.33$. Authors vary in their opinions as to the size of a correlation coefficient necessary to be considered important; however, when we consider that the standard deviation of the observed y 's (hemoglobin) is 1.38 and that of the y 's predicted by aid of the associated x 's (red blood cells) is 1.33, the value of information regarding the erythrocyte count of an infant is essentially valueless as an aid in making any statement as to what the hemoglobin content of his blood should be. The fact that r_{xy} (and consequently m) is positive, although small, does say that an increase in the red blood cell count is usually accompanied by a slight increase in the amount of hemoglobin.

A geometrical interpretation of the hemoglobin-leucocyte correlation, Table II, would seem to tell a very similar story to that of Table I. These data presented in a table in which the frequency class interval for the white blood cell counts is taken as three units (which was originally done with essentially the same results, but offered difficulties in presentation) instead of six as in this table, makes the independence of these two sets of measurements much more in evidence. For these data we find $r_{yz} = 0.07$, $P.E. r_{yz} = 0.02$, $\frac{\sigma_y}{\sigma_z} r_{yz} = 0.00$, and $\sigma_e = 1.38$. The constants show the distinct lack of any linear dependence of the amount of hemoglobin upon the number of white blood cells.

The scatter of the entires in Table III also pictures a lack of quantitative dependence of the two types of blood cells measured by this table, namely, the red and the white blood cells. Graphically this table also makes a clearer presentation when the leucocyte class interval is taken as three instead of six, but the numerical value of the statistical constants indicates sufficient directness.

Here $r_{xz} = 0.13$, $P.E. r_{xz} = 0.02$, $\frac{\sigma_z}{\sigma_x} r_{xz} = 0.06$, $r_{xz} = 0.06$, and $\sigma_e = 29.14$. From so large a number of measurements as we have here, 1,644, upon which to base our conclusions, the slight increase in the leucocyte count accompanying a given increase in the erythrocyte count is negligible in value for predictive purposes. With $\sigma_z = 29.40$ and $\sigma_e = 29.14$ any information gained from a knowledge of the number of red blood cells of an infant offers us no help in estimating his white blood cell count.

If we evaluate the multiple correlation coefficient on the assumption of y being expressible as a linear function of both x and z , say $y = f(x, z)$, we find that $r_{xyz} = 0.27$. The standard error of the y 's (hemoglobin values) as predicted from the corresponding x 's and z 's is found to be 1.33. So again, remembering that $\sigma_y = 1.38$, we are in no better position to estimate the value of the hemoglobin of a given individual knowing both his red blood cell and white blood cell counts than if we had no information concerning them. A similar statement can be made about any one of the three quantities with respect to the other two.

Of the three constituents under consideration, probably the most often associated in discussion are the red blood cells and the hemoglobin. From Table I, which represents the data of these measurements, we find $r_{xy} = 0.26$, $P.E. r_{xy} = 0.02$, $\frac{\sigma_y}{\sigma_x} r_{xy} = 0.01$, and $\sigma_e = 1.33$. Authors vary in their opinions as to the size of a correlation coefficient necessary to be considered important; however, when we consider that the standard deviation of the observed y 's (hemoglobin) is 1.38 and that of the y 's predicted by aid of the associated x 's (red blood cells) is 1.33, the value of information regarding the erythrocyte count of an infant is essentially valueless as an aid in making any statement as to what the hemoglobin content of his blood should be. The fact that r_{xy} (and consequently m) is positive, although small, does say that an increase in the red blood cell count is usually accompanied by a slight increase in the amount of hemoglobin.

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Weight—Friedberger and Guwitz⁷ found serums from 150 to 600 gm guinea pigs showed marked differences in complement content, wholly independent of age and weight. Kolmer⁸ did not find any variation in hemolytic activity between a group of 200 to 280 gm pigs and another group of 400 to 580 gm, but did recommend the heavier animals, as serums of younger ones were more likely to be anticomplementary. Cummer⁹ recommended pigs weighing 400 gm or more, because small pigs yielded little blood and that work in complement.

Sex—For satisfactory complement Kolmer⁸ recommended the use of large, well nourished animals, males preferred, which had not been fed within twelve to twenty four hours of bleeding. He also avoided using pregnant animals, despite the fact that he found little difference between their titer and males of the same weight, and suggested that pooled serums of three or more pigs should be used. Cummer⁹ advised that pregnant animals be avoided and pooled serums of three or more pigs be used, since there is considerable variation between different pigs. Wadsworth¹⁰ stated that three, and preferably six or more pooled serums are desirable, and to avoid chylous serum advised not bleeding until several hours after feeding. Simmons¹¹ suggested that blood for complement be obtained from three or more full grown, healthy male guinea pigs. Although giving no reason, he stated that females should be avoided because of the possibility of pregnancy. A deficiency in complement was associated with pregnancy according to Brown¹². Rich¹ found that neither castration nor pregnancy affected the complement content of guinea pigs. Gladwohl¹ merely stated that complement varied from time to time in animals and was present in particularly large amounts in guinea pigs. Wadsworth, Harris, and Gilbert¹³ stated that healthy animals, preferably males, should be tested for hemolytic activity and fixability, and pooled serums of three or more satisfactory animals should be used. The same statement also appeared in a report by Gilbert.¹⁴

Feed—Simola and Bruns¹⁵ reported that neither A nor C avitaminosis nor the administration of vitamin D in the form of irradiated ergosterol to guinea pigs had any effect on complement. With rats on a diet deficient in vitamin A, Osborn⁶ observed lower titers than with controls receiving cod liver oil. While not bearing directly on complement, Madsen, McCay, and Maynard¹⁶ found it difficult to raise guinea pigs using a synthetic diet free from hay and grain but including cod liver oil or vitamin A D concentrate. Hilgers and Zam¹ stated that acid and alkali diets generally have no effect on complement titer.

A report of the United States Department of Agriculture¹⁸ recommended that guinea pigs be given water, salt, dry grains, hay, and a daily feed of green stuffs. Rich,¹ using many hundreds of guinea pigs and feeding a variety of foods over a period of years, could find little evidence that diet had any effect on complement. Kolmer,⁸ although admitting the lack of systematized and scientifically regulated diet experiments, stated that a mixed diet maintaining animals in good health and normal weight was sufficient to yield acceptable complement. In order to prevent dilution of blood by hydrops, Cummer⁹ recommended that pigs have a diet of oats only for at least a day before bleeding. Austin,¹⁷ working on the nonspecific fixability of guinea pig serum with Bordet's antigen, kept animals on a diet of oats, cabbage, and alfalfa, fed dry in winter and green in summer. Browning⁴ stated, based on the work of Griffith and Scott, that in guinea pigs

TABLE I

A COMPARISON OF COMPLEMENT TITERS, AGE, AND WEIGHT OF GUINEA PIGS HOUSED OUTDOORS AND INDOORS ON DIFFERENT RATINGS
(Results of titration expressed as e.c. of 1:10 complement required to hemolyze 0.5 e.c. of sheep cells with 2 units of hemolysin [0.5 e.c. of 1:2,000 dilution])

NUMBER AND LOCATION	CAGE	GUINEA PIG				AGE, TITER, AND WEIGHT												
		NUMBER	LITTER NUMBER	SEX	WEIGHT 6/4	1935					1936							
	FEED					6/14	6/29	7/30	8/31	10/2	11/1	12/3	12/31	1/30	2/29	3/28	5/2	6/6
1 Outside	Wheat	409	1	M	90	0.18	0.24	0.22	0.18	0.22	0.18	0.10	0.10	0.12	0.10	0.08	0.12	0.14
	Oats					95	120	231	356	459	532	545	535	576	622	660	653	641†
	Greens	198	9	M	274	0.18	0.18	0.22	0.24	0.20	0.16	0.10	0.10	0.10	0.08	0.08	0.10	0.10
		408	10	M	90	272	302	428	554	673	762	772	775	862	947	930	1002	1013
									0.26†	0.26†	0.20	0.18	0.10	0.12	0.10	0.12	0.10	0.10
		410	3	F	104	0.26†	-	0.22	0.16	0.16	0.20	0.14	0.16	0.24	0.18	0.26	0.26†	0.26
		187	4	F	142	105	122	232	376	502	637	611	654	684	704	763	1004	710
		177	1	M	92	0.16	0.16	0.20	0.20	0.22	0.18	0.22	0.12	0.22	0.12	0.26	0.18	0.24
1 Inside	Wheat					144	164	259	355	428	506	628	494	533	633	706	598	646
	Oats					0.22	0.24	0.22	0.20	0.16*								
	Greens	194	7	M	136	104	120	244	370	457								
		301	12	M	81	0.22	0.20	0.22	0.22	0.24	0.16	0.16*						
						160	198	336	448	526	600	626						
									0.26†	0.26†	0.18	0.10	0.08*					
		406	4	F	190	0.16	0.18	0.16	0.18	0.26†	0.20	0.20	0.26	0.20	0.14	0.12	0.10	0.20
		191	6	F	86	203	236	368	533	637	558	595	718	846	937	1054	1062	1101
2 Outside	Pellets	418	2	M	95	0.24	0.24	0.22	0.20	0.22	0.14	0.12	0.16	0.26†	0.12	0.12	0.18	0.14
	Oats					98	119	260	418	553	468	535	726	639	636	710	764	825
						0.20	0.20	0.18	0.20	0.20	0.18	0.10	0.10	0.08	0.10	0.10	0.12	0.14
						123	184	327	470	556	608	638	624	612	658	713	715	668

*Died.

†Weight expressed in grams.

Note: Large increases in weight attended by subsequent fall in weight in females was due to pregnancy.

nutrition had no definite relation to complement. Ruediger³ found little difference in the titer of guinea pigs fed during the winter on various mixtures of vegetables in addition to a basic diet of bread, hay, and oats.

Temperature and Sunlight.—Little information has been available on the effect of sunlight and temperature upon guinea pigs. A temperature below 65° F. was unsuitable for guinea pigs according to a report of the United States Department of Agriculture.¹⁸ Remlinger and Bailly²⁰ found that exposure of rodents to the sun for only half an hour would cause death, and concluded that their susceptibility was probably due to habitual sheltered existence.

EXPERIMENTAL

Preparation of Materials.—Guinea pigs were lightly etherized and 2 c.c. of heart's blood withdrawn for complement. This was placed in a sterile agglutination tube, slanted, allowed to stand at room temperature for one hour and then placed in a refrigerator (7° to 9° C.) for twenty-four hours before the titer was determined. Each sample was centrifuged to obtain clear serum. The same lot of hemolysin, obtained from Difco Laboratories, was used throughout the experiments, and was titered before each use.

Corpuscles were obtained from a ewe, blood from the jugular vein being aseptically aspirated into a 30-c.c. syringe, immediately expelled into a sterile flask containing glass beads, shaken for about ten minutes to defibrinate the blood, and refrigerated. The corpuscles were washed the same day, three times or more, packed by centrifuging for twenty minutes, made up to a 50 per cent suspension, refrigerated and used within two days. The corpuscles were made up to a 2 per cent suspension in saline for use in the tests. Throughout the work all dilutions were made with day-old saline only,²¹ prepared from 8.5 gm. of C. P. sodium chloride, made up to 1,000 c.c. with cold tap water, and heated in flowing steam for thirty minutes.

Complement Titration.—The complement was titrated by determining the least amount necessary to bring about complete hemolysis of 0.5 c.c. of a 2 per cent suspension of sheep cells in the presence of two units of hemolysin contained in 0.5 c.c. The complement was diluted 1:10 and varying amounts were used, ranging from 0.06 to 0.26 c.c., with 0.02 c.c. intervals. The total volume was made up to 3 c.c. with saline, hemolysin, and complement controls set up for each titration and titers recorded after a one-hour incubation in a water bath at 37° C. All mention made hereafter of complement titers refers to dilutions of 1:10.

Animals.—Nine different litters of guinea pigs were received on June 4, 1935, and three more litters were added later to replace animals that had died. The variations in weight as listed in Table I show they varied somewhat in age, only litters 5 and 9 apparently being over three weeks old, since pigs at birth vary from approximately 40 gm. to 90 gm.

Distribution.—The animals were distributed in various cages as shown in Table II, so comparisons could be made of litter mates reared under various conditions. Three cages were placed outdoors to determine any effect of sunlight and temperature, these remaining outside regardless of weather, except from

January 23 to February 26, 1936, when unusually heavy and continued snowfall made it necessary to move them to an unheated barn. Three cages were kept in doors in a steam heated animal house where the temperature could be regulated throughout the year.

The animals were placed in rectangular cages 3 by $1\frac{1}{2}$ by 1 foot deep, set on legs $1\frac{1}{2}$ feet high. The sides of the cages were covered with fine mesh wire nailed to a wooden frame. The top, covered with the same type of wire, was hinged so that it could be raised. The floor was constructed of wood over which a heavy, removable, galvanized iron tray was placed. In the outdoor cages one half of each, including top, sides, and corresponding end, was covered with heavy tar roofing paper to protect the animals from the weather. A small wooden box, one end of which had an opening, was placed under the covered end to give additional protection. The other half of the cage remained open for the admission of sunlight.

Rations—Guinea pigs placed in the three outdoor and three indoor cages were maintained entirely on one of the following three rations commonly used, fresh water being available at all times. Ration 1 was a grain mixture of wheat and oats, with greens (cabbage, kale, lettuce, grass, or spinach) fed each day. Ration 2 contained oats and a pellet food consisting of wheat germ, soy bean oil meal, corn germ meal, alfalfa leaf meal, ground oats, wheat middlings, yellow corn meal, blackstrap molasses, calcium carbonate, and iodized salt. Cabbage was also fed daily as a source of vitamin C. Ration 3 was a commercially prepared, ready mixed product, consisting of chopped alfalfa, crushed oats, yellow cracked corn, blackstrap molasses, and a pellet feed composed of wheat germ meal, soy bean oil meal, corn germ meal, wheat middlings, calcium carbonate, and iodized salt. Cabbage was fed with this ration on alternate days. The guinea pigs were fed and watered in the early morning each day, and the cages were cleaned and bedded twice a week.

RESULTS

The first complement titration was made on June 14, 1935, another two weeks later, and the animals were bled (feed being removed for eighteen to twenty four hours before bleeding), and serums titrated monthly thereafter through June 6, 1936. Weights were recorded each week, but are shown in Table I only at the time of each titration.

Data are available on twenty five of the twenty eight animals used during this work. Of fifteen males four died before the completion of the experiment, no data being collected on one, and of thirteen females three died without information being obtained on two.

Sex—In all cages, regardless of diet, the blood serum of male guinea pigs gradually increased in titer between the third and sixth month. Of animals surviving until completion of the test period, however, five (distributed in four different cages) showed some slight drop in titer during the last month or two (May and June), the titer of six remained about equal to the highest titer reached, while three that died before completion of the experiment showed an increased titer. As shown in Table I, the weights of these animals could not be correlated

TABLE 11

A COMPARISON OF COMPLEMENT TITERS, AGE, AND SEX OF GUINEA PIG LITTER MATES

(Results of titration expressed as e.e. of 1:10 complement required to hemolyze 0.5 e.e. of sheep cells with 2 units of hemolysin [0.5 e.e. of 1:2,000 dilution])

GUINEA PIG			DATE OF DETERMINING TITER														
LITTER NUMBER	SEX	NUMBER	CAGE NUMBER AND LOCATION	1935							1936						
				6/14	6/29	7/30	8/31	10/2	11/1	12/3	12/31	1/30	2/29	3/28	5/2	6/6	
1	M	409	1, outside	0.18	0.24	0.22	0.18	0.22	0.18	0.10	0.10	0.12	0.10	0.08	0.12	0.14	
	M	177	1, inside	0.22	0.24	0.22	0.20	0.16*									
	F	178	2, outside	0.26+	0.26+	0.18	0.24	0.12	0.16	0.16	0.20	0.12	0.26	0.24	0.20	0.26+	
2	M	418	2, outside	0.20	0.20	0.18	0.20	0.20	0.18	0.10	0.10	0.08	0.10	0.10	0.12	0.14	
	F	180	2, inside	0.22	0.20	0.12	0.14	0.14	0.24	0.12*							
	F	181	3, outside	0.26+*													
3	F	410	1, outside	0.26	-	0.22	0.16	0.16	0.20	0.14	0.16	0.24	0.18	0.26	0.26+	0.26	
	F	183	3, outside	-	0.24	0.22	0.24	0.24	0.18	0.20	0.20	0.20	0.24	0.22	0.26+	0.12	
	F	184	3, inside	0.24	0.20	0.22	0.24	0.24	0.26+	0.18	0.18	0.18	0.20	0.22	0.24	0.26	
4	M	186	2, inside	0.20*													
	F	406	1, inside	0.16	0.18	0.16	0.18	0.26+	0.20	0.20	0.26	0.20	0.14	0.12	0.10	0.20	
	F	187	1, outside	0.16	0.16	0.20	0.20	0.22	0.18	0.22	0.12	0.22	0.12	0.26	0.18	0.24	
5	M	189	2, inside	0.24	0.20	0.18	0.18	0.22	0.16	0.16	0.22	0.10	0.10	0.08	0.08	0.12	
	F	403	2, outside	0.20	0.16	0.20	0.20	0.20	0.12	0.12	0.22	0.10	0.20	0.22	0.24	0.26	
	F	190	3, inside	0.26+	0.26+	0.12	0.22	0.24	0.24	0.26	0.26+	0.22	0.20	0.22	0.26+	0.20	
6	M	415	3, inside	0.24	0.20	0.10	0.10	0.12	0.14	0.10	0.10	0.08	0.10	0.10	0.10	0.08	
	M	401	3, outside	0.26+	0.24	0.24	0.22	0.20	0.10	0.10	0.08	0.08	0.10	0.10	0.08	0.12	
	F	191	1, inside	0.24	0.24	0.22	0.20	0.22	0.14	0.12	0.16	0.26+	0.12	0.12	0.18	0.14	
7	M	194	1, inside	0.22	0.20	0.22	0.22	0.24	0.16	0.16*							
	M	195	2, inside	0.20	0.24	0.20	0.24	0.24	0.16	0.16	0.14	0.10	0.10	0.10	0.14	0.16	
	M	413	2, outside	0.22	0.22	0.20	0.20	0.12	0.16	0.14	0.10	0.14	0.12	0.12	0.12	0.10	
8	F	422	3, outside	0.26+	0.24	0.12	0.14	0.12	0.16	0.26	0.12	0.22	0.22	0.12	0.24	0.20	
	M	198	1, outside	0.18	0.18	0.22	0.24	0.20	0.16	0.10	0.10	0.10	0.08	0.08	0.10	0.10	
	F	199	3, inside	*													
10†	M	408	1, outside				0.26+	0.26+	0.20	0.18	0.10	0.12	0.10	0.12	0.10	0.10	
11†	M	316	3, outside				0.20	0.16	0.14	0.10	0.08	0.08	0.08	0.08	0.06	0.06	
12†	M	420	2, inside				0.24	0.20	0.16	0.14	0.12	0.12	0.12	0.10	0.12	0.10	
	M	301	1, inside				0.26+	0.26+	0.18	0.10	0.08*						

*Died.

†Litters 10, 11, and 12 were added August 31, 1935, to replace animals that died.

with the slight drop in complement strength, which agrees with the considerable individual variation often reported previously in mature animals

The females almost invariably showed a considerably lower titer than males, beginning between the fourth and sixth month, although their original complement content might have been equal or better*. This usual loss of hemolytic activity was probably due to pregnancy, a factor discounted by certain investigators. An examination of Table I shows this lowering of complement content during pregnancy. Larger increases and decreases in weight, due to pregnancy and parturition, were more evident in the weekly weights recorded than in the monthly weights shown in Table I. Because females will almost invariably breed as soon as the young are delivered, after the first several months they were nearly always pregnant. The titers of the females while usually poor during pregnancy, were lowest near the end of their terms. Frequently when (Table I) a litter had been born as indicated by the weights, and the female had not begun to show signs of another pregnancy, the titers were considerably higher.

Because of this interference of pregnancy with complement titer, few, if any, conclusions could be drawn comparing litter mates or regarding the influence of feeding, sunlight, temperature or weight on females.

Age and Weight—Guinea pigs reach maturity in from four to five months according to a report of the United States Department of Agriculture,¹⁸ but, as seen in Table I, the maximum weight was not reached at that time. Whenever the complement of male animals increased to 0.16 cc, they had also reached a weight of 445 gm or more (Table I) except three animals of different litters, housing and diet giving this titer at 336 to 376 gm. Only two (of the three above exceptions) pigs reached a titer of 0.1 cc before weighing 525 gm, and of all males giving this titer eight or 57 per cent, weighed at least 600 gm.

Young male animals did not have a titer of 0.14 cc or better until approximately four months or older and the majority were between five and seven months old before their titer reached 0.1 cc. Animals No. 316 and No. 415 were exceptions, showing equivalent or better titers in three and two months, respectively. As difficult as it was to get data on female guinea pigs, what has been indicated about the males, in general, held true for females.

Litter Mates—In considering female litter mates raised in different cages, it is difficult to obtain significant results largely because of the interference of pregnancy with complement activity. Additional complications arose where litters contained both males and females. However, some information may be obtained by a comparison of litters 3 and 4 in Table II. All of these animals were females except one male in litter 4 that died after the first bleeding. Two of these in litter 4 had a higher initial titer than those of litter 3 and tended to maintain this throughout the experiment. One in each litter was raised in cage 1 inside where identical conditions of maintenance did not seem to equalize their titers. Complement titers of all animals were low at the beginning but variations among the same litters were noticeable. Litter 5, No. 403, had a higher titer than No. 190 for nine bleedings, twice was of equal titer and twice lower.

*Exception occurred in cage 1 inside where all male animals died at least four months before the experiment was completed and in cage 3 outside where one female did give a decidedly higher titer at the last bleeding.

In comparing female and male litter mates, the males invariably reached and maintained a higher titer, although this comparison may be unfair because of pregnancy. One female of litter 6 (No. 191) lived at least the last four months without being pregnant, and her titer never equalled that of the two males of the same litter.

Only in litter 6 did two male litter mates live to the completion of these studies, and after six months their complement strengths were nearly parallel. In litter 7, where seven comparable titers of two males were obtained, the titers again paralleled one another. All members of both litters (6 and 7) were raised under different conditions (Table II). Definite conclusions regarding male litter mate comparisons can hardly be reached because the number of comparisons were too few. All animals came from good stock and all males had reached a titer of 0.1 c.c. by the time they had been on test eight months or less.

Feed.—In preparing the three diets, no attempt was made to feed deficient food, but rather to use rations successfully used in laboratories where pigs are raised as a source of complement. Data on females on the various rations are again uncertain for reasons already discussed. As indicated in Table I, a titer of 0.12 c.c. was obtained only twice, both in the same pig, on ration 1, housed outdoors, and in only one instance on the same ration indoors. On ration 2 outside, the two females had two titers each of 0.12 c.c., and once one had a titer of 0.10 c.c. at a time when she had just previously given birth to young. The one female on ration 2 inside twice had a titer of 0.12 c.c. One female on ration 3 outside had a titer of 0.12 c.c. four times, and a cage mate, only once. On ration 3 inside a titer of 0.12 c.c. was reached only once.

On ration 3, one male of litter 6 indoors and one of this litter outdoors, and another male outdoors reached a titer of 0.10 c.c. by the second, fifth, and fourth month respectively. Males on ration 2 took longer to reach such titers, two indoors requiring eight months, and the two outside, six and seven months. The three males on ration 1 inside all died before the experiment was completed, only one having a titer of 0.10 c.c., reaching this in four months. The three males on ration 1 outside acquired a titer of 0.10 c.c. by the sixth month.

Apparently males fed ration 3 showed some slight superiority over those fed other rations. We would favor this ration because it is well balanced, easily handled, reasonably cheap, and necessitated green feed only every other day, and because all males on this diet showed a high titer in a reasonable time.

Sunlight and Temperature.—These factors apparently did not have any appreciable effect on complement titer of male and female guinea pigs. However, males in cage 2 inside required a little longer to reach a satisfactory titer than any others, while the male in cage 3 inside was the first to reach a titer of 0.1 c.c. The males in cage 3 outside reached a satisfactory titer a little in advance of the other outside cages.

Animals housed outdoors were kept there regardless of the weather. The young animals did very well through the hot summer months when temperatures of 100° F. were not uncommon. However, they preferred the shade and thus cannot be compared to the results of Renlinger and Bailly²⁰ who found rodents may die if left in the sun for as short a period as half an hour. During December, 1935, and January and February, 1936, the temperature was

usually near freezing, and subzero weather with unusually heavy snowfall was not uncommon. For this entire period the guinea pigs were kept outdoors except from January 23 to February 26, when they were housed in an unheated barn. This extreme weather had no effect upon complement as the titers (Table I) were always equal to and sometimes better than in the corresponding cages inside kept at a rather uniform temperature throughout the year.

After this work was completed, the temperature reached 104° F with high humidity prevalent, between July 6 and July 10, 1936, and six of the animals died in the outdoor group that had been allowed to stay outside and treated as though on experiment, while no deaths occurred indoors. Postmortem examinations revealed only congested lungs, probably due to rapid respiration, indicating that the pigs had died of suffocation, probably as a result of intense heat and high humidity. Had the pens been larger with more space for the circulation of air, this might not have happened. A year before the young animals were exposed to heat as intense as that encountered in 1936, but were apparently able to withstand the excessive heat better than the mature animals.

It is noteworthy that wide differences in temperature had no effect upon complement strength, although it is not recommended as a result of this work that animals be reared in outdoor pens. The animals did survive extreme cold very well, and with larger and better aerated pens, sheltered from the sun, could probably have survived the excessive heat although there are insufficient data upon this point.

DISCUSSION

The effect of sex of guinea pigs upon complement titer could not be accurately determined from these experiments, largely due to pregnancy radically lowering titers. This effect of pregnancy differs from Rich,¹ who reported pregnancy did not affect titer, and from the results of Kolmer,⁸ confirms the results of Griffith and Scott quoted by Browning,⁴ and justifies the recommendations of Kolmer,⁸ Cummer,⁹ Simmons,¹¹ and others.¹³⁻¹⁶

As the guinea pigs became older the complement titer of males greatly increased, six of eleven living until completion of the studies showed no drop in titer during the last several months, and the drops manifested by five were probably individual variations, since their distribution could not be correlated with other factors. This increased titer of mature males is contrary to the report of Hyde,² but agrees with the work of Osborn⁶ on rats. Our results showing low titers of young animals also confirmed by repeated examination of other week-old guinea pigs, might be considered to agree with Friedberger and Gurwitz⁷ who reported that newborn animals may have the same titer as the mother, since, at the time of birth of the young, the mothers usually had a very low titer. Findlay, Fua, and Noeggerath, according to Browning,⁴ found a marked increase during the first three months of life, which our results substantiate, although we found most males must be five to seven months old before reaching a titer of 0.1 c.c. even though some animals reached that titer at three or four months.

Guinea pigs should weigh not less than 450 gm and preferably 600 gm or more before the most potent titer is reached according to our results. Cummer's⁹ recommendations that guinea pigs weigh 400 gm or more before use for complement compare closely with our results. The use of larger animals is indi-

cated however and Simmons¹¹ definitely stated that full-grown healthy males should be used. The authors' results are somewhat contrary to Friedberger and Gurwitz⁷ who found that differences in complement titer of animals between 150 and 600 gm. were independent of age and weight. Kolmer⁸ found little difference in hemolytic activity between a group of 200- to 280-gm. animals and another averaging 400 to 580 gm. when fed, bled, and the serums treated alike; but since the sex and titer were not given, our results could not be reconciled.

The recommendation of the United States Department of Agriculture¹⁸ and Austin¹⁹ were followed in that diets were well balanced. Rich,¹ Reudiger,³ and Browning⁴ were of the opinion that diet had little effect upon complement content. Kolmer⁸ stated that a mixed diet, maintaining animals in good health, was sufficient to yield acceptable complement, and in general, we reached the same conclusion after feeding animals for a year. Simola and Brunius¹⁵ reported that neither A or C avitaminosis nor the feeding of vitamin D would affect the potency of guinea pig complement. The present work cannot be compared with that on vitamins, but the results would tend to show the probable lack of effect of feed on complement.

Guinea pigs are generally raised indoors. A report of the United States Department of Agriculture¹⁸ points out that the temperature should not drop below 65° F. For no apparent reason, the majority of the animals which did not survive the experiment were housed indoors. The outdoor group all survived exposure for some time to subzero weather, and when young, survived extreme heat in summer. As far as complement titer was concerned little difference was noted between titers of those raised in outdoor pens and indoors.

CONCLUSIONS

Young guinea pigs consistently had low, unsatisfactory titers.

All male guinea pigs gradually increased in complement titer between the third and sixth month, while females almost invariably had a lower titer than males after the fourth to sixth month due to pregnancy. This interference of pregnancy, usually not considered by others, made it difficult to reach conclusions on litter mates, age, weight, feeding, sunlight, and temperature.

Male animals did not usually reach a titer of 0.1 c.e. until five to seven months old, and this titer was not reached by 57 per cent of the males examined until they weighed 600 gm. or more.

Comparisons of litter mates were also obscured by pregnancy, variation occurring in females of the same litter as well as between litters. In limited observations on males, the titers within the same litter agreed rather closely, with the usual differences between litters. With such variations between individual females of the same litter and different litters and so few comparisons of males, any significance of litter mates could not be evaluated.

Complement titers of guinea pigs maintained for more than a year upon one of three commonly used rations indicated the probable lack of effect of a satisfactory diet upon complement, although males reached a good titer earlier upon the complete commercial feed supplemented by cabbage every other day.

The complement titer of guinea pigs reared and kept outdoors was not affected by extreme cold during winter. Likewise the titer was not influenced in summer, although many mature animals died from suffocation during excessive heat and humidity.

Our results suggest that the most satisfactory complement should be obtained from male guinea pigs, usually not less than five to seven months old, ordinarily weighing 600 gm or more, and that pregnant animals are undesirable sources of complement.

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LABORATORY METHODS

DIFFRACTION METHODS FOR MEASURING THE DIAMETER OF THE RED BLOOD CELL²

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THE measurement of the size of the erythrocyte is a valuable procedure in a study of the blood dyscrasias.¹⁻³ The determination of the mean erythrocyte volume is a simple test and has become a part of the routine blood study in many laboratories. The mean erythrocyte volume affords the most satisfactory basis for a laboratory classification of the anemias, and often gives information concerning etiology and the effect of treatment.

The determination of the diameter of the erythrocyte as a measure of the size of the erythrocyte has been advocated by many workers. Price-Jones,⁴ in numerous publications, has demonstrated the clinical value of the distribution curve of the erythrocyte. By measuring a large number of cells (200 to 1,000), the range of diameters, which indicates the degree of anisocytosis as well as the mean diameter, is obtained. Price-Jones projects the cells with a camera lucida at a known high magnification and measures the image directly with a millimeter rule. Most workers have used an ordinary micrometer eyepiece. I have employed a filar micrometer as the movable hair facilitates measurement. Another very satisfactory method is the direct projection of the blood film on the ground glass plate of a photomicrographic camera at a fixed high magnification and direct measurement of the image with a millimeter rule. The measurement of a sufficiently large number of cells to give an accurate determination of the mean diameter and to construct a distribution curve is a laborious and time-consuming procedure. This has prevented any extensive use of direct measurement for determining cell size. The volume of the cell is also a much more sensitive indicator of cell size than is the diameter. This is well illustrated by the findings in pernicious anemia. Here the average increase in diameter of 14 per cent corresponded to a 46 per cent increase in volume.

The cell diameter is an accurate indicator of cell volume provided any variation in cell morphology affects diameter and thickness equally. In pernicious anemia and in some other clinical states, this is usually true. In the microcytic hypochromic anemias, however, there may be no reduction or even an increase in diameter with a marked decrease in volume. This can be explained only on the basis of a great decrease in thickness. In congenital hemolytic icterus, there is characteristically a dissociation of the normal relation of diameter and volume since the cells tend to be spherical and so are much thicker than normal. In such conditions, there is a direct relation of the tend-

*From the Cleveland Clinic.

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ency to spherocytosis to the characteristic increased fragility in hypotonic salt solutions."

Knowledge of the mean cell diameter, however, is a valuable addition to the blood study in many cases and in certain conditions is essential. If both the volume and diameter are known, the thickness can be calculated and a cross section of the mean cell visualized. In order to make the determination of the cell diameter a simple, rather than a laborious procedure, the diffraction method has been utilized. The availability of the diffraction method of measuring erythrocytes depends on the fact that a blood film placed in a beam of polychromatic light breaks it up into successive bands of its component colors. The red cells act as opaque disks on a screen just as do the apertures of a diffraction grating. The size of the halo depends on the diameter of the red cells and its distance from the light. It varies inversely as the diameter of the erythrocytes and also inversely as its distance from the light. Thomas Young, in 1813, first showed that the principle of diffraction could be used to measure small objects. He constructed an instrument which he called an *eniometer* or wool measurer and with which he measured red cells, wool hairs, and vegetable spores. No practical application of Young's discovery was made until Pijper independently rediscovered the application of the principle of diffraction to the measurement of small objects. It is due largely to the work of Pijper that diffraction methods have been made practical and popular. Recently Pijper's instrument has been manufactured by Zeiss as "*Pijper's blood cell tester*." This apparatus has a built-in source of light which requires no adjustment. There is a horizontal stage on which two blood smears can be placed. The diffraction patterns appear on a ground glass screen which forms the top of the apparatus. A partition is placed in the middle of each diffraction pattern so the remaining semicircles appear in close apposition on the screen, thus facilitating comparison. Each diffraction pattern consists of a bright center surrounded by an orange ring and then follow the colors of the spectrum in natural order, starting with red. After that green and red encircles alternate with one another. The mean diameter is determined by measuring the diameter of the yellow ring. The greater the number of macrocytes, the smaller and more intense the violet ring. An increase in microcytes results in a larger red ring. The diffraction pattern thus shows not only the mean diameter but also the anisocytosis and the frequency of macrocytes and microcytes. Direct measurement of the violet ring is made from the inner edge and of the red ring from the outer edge. The distance between the violet and red rings measures the degree of anisocytosis.

Emmons, in 1927,² described a simple instrument based on Young's method for measuring the mean diameter of erythrocytes. It consists essentially of two telescopic tubes. The larger, 10 inches in length, is fitted with two small batteries and with electric bulb and switch like an ordinary flashlight. Immediately in front of the electric filament is placed a disk perforated near its periphery by a ring of holes and with one hole at its center. By this arrangement, direct illumination is obtained through the central hole and an indirect and consequently dimmer light from the holes at the periphery. The other tube, which telescopes completely into the first, carries the blood slide and is

graduated to read off directly the diameter of the red cells on the film. Emmons designated this instrument the eriometer, using the name given by Thomas Young to his instrument employing the same physical principles. Unfortunately, this instrument has never been made available commercially. To operate the instrument, a blood film is placed in the slot provided for it. The telescopic tube is adjusted to make the red halo overlies the peripheral ring of holes. The diameter of the cells is then read off directly from the scales on the inner tube.

Eve, in 1929,¹⁰ made an instrument for measuring the diameter of red cells which he called the "halometer." This instrument also is based on the diffraction principle.

Boek, in 1933,¹¹ described an "erythrocytometer" for measuring red cells, again employing the principle of diffraction. In this instrument, the beam of light passes through two apertures so a blood film placed in the beam gives two spectra. A sleeve is adjusted until the outer red bands of the two spectra touch tangentially, and the cell diameter is read off directly from a scale on the supporting tube. Pijper¹² criticizes Boek for using the red spectral ring for measurement since, he says, this is an expression of the smallest cells in the blood film as is easily demonstrated with his apparatus. The red halo is satisfactory for measuring the mean diameter of normal films since the anisocytosis is relatively slight, but in the anemias the anisocytosis might be marked, so the size of the red circle should not be used to measure the mean diameter. This criticism of Pijper probably explains the unsatisfactory readings we have often obtained with the Boek erythrocytometer in pernicious anemia and the extreme microcytosis of congenital hemolytic icterus.

Diffraction methods for measuring erythrocytes have also been described by Millar¹³ and Falisi.¹⁴ None of the diffraction methods necessarily give the true mathematical average of cell diameters. The intensity of a halo at any given point is directly proportional to the number of cells of the corresponding diameter, so the point of maximum intensity indicates the mode or characteristic diameter of all the cells present. The mode gives as much information, however, as does the true mean diameter.

In 1931,¹⁵ I reported a comparison of measurements made with the Emmons eriometer and with a filar micrometer. I also calculated the diameter in each instance from the mean cell volume using Warburg's nomogram,¹⁶ which is correct only when the relation between diameter and thickness remains constant. I concluded that the value of determining the mean diameter did not justify the time required for the procedure when direct measurement is employed. The readings obtained with the Emmons instrument checked closely with those obtained by direct measurement. The eriometer readings were made on the same stained film used for the filar micrometer measurements.

It has become increasingly evident that measurement of the mean diameter of the red cell is of value if not too time-consuming. Thus the fragility of the red cell in congenital hemolytic jaundice has been shown to be dependent on the shape of the cell, which can be determined only when the diameter as well as the volume is known.³ I have compared readings made on the same film by Emmons', Pijper's and Boek's methods with direct measurement and with calculation of the diameter from the volume readings. The results show that very ac-

curate readings are obtained with the Emmons curometer and the Piper blood tester. The readings with the Bock erythrocytometer are not constantly accurate in the abnormal cases. The Emmons instrument is unfortunately not available on the market. We now use this instrument routinely in all blood studies since no dark room is required, it is portable, and gives accurate readings.

Piper⁸ suggests that his instrument be used qualitatively as well as quantitatively. Since an abnormal film can be compared directly with a normal blood film, one may detect a variation from normal immediately. He also emphasizes the importance of the red and violet rings in detecting anisocytosis and the relative number of large cells and small cells. He differentiates three types of patterns.

Type I. Violet red and yellow rings, all small. Distance between violet and red rings relatively large. This pattern means macrocytosis and pronounced microcytosis typical of pernicious anemia.

Type II. Yellow ring of normal diameter which means normal mean diameter of cells. Violet ring is smaller, and red ring is larger than normal, indicating more anisocytosis than normal. The distance between the violet and red rings measures the degree of anisocytosis.

Type III. All rings larger than normal and distance between violet and red rings often relatively increased. This is the typical pattern of congenital hemolytic icterus (spherocytic anemia) and the microcytic anemias due to iron deficiency.

TABLE I

COMPARISON OF DIFFERENTIOMETER WITH DIRECT MEASUREMENTS OF STAINED FILMS OF NORMAL BLOOD

NO	MEAN CELL VOLUME BY HENVIATO CRET	MEAN CELL DIAMETER BY					CALCULATED MEAN CELL THICKNESS	VOLUME THICKNESS INDEX
		DIRECT MEASUREMENT	EMMONS CROMETER	PIPER BLOOD CELL TESTER	BOCK ERYTHROCYTOMETER	CALCULATION FROM MEAN CELL VOLUME		
	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	
1	92	76	76	71	76	77	205	1.06
2	89	73	74	69	73	76	215	1.15
3	91	75	77	71	75	77	210	1.06
4	90	78	76	71	78	77	190	0.96
5	90	80	81	74	80	77	185	0.90
6	92	76	76	72	76	78	215	1.06
7	89	74	75	70	74	77	210	1.12
8	88	77	76	71	77	76	190	0.95
9	87	76	77	72	76	75	190	0.99
10	96	80	77	72	80	78	190	0.95
Average	90	76	76	71	76	77	200	1.02

In making the comparative measurements, I have measured only the yellow ring to determine mean cell diameter in the Piper blood cell tester. The diameter of the cells in a blood film may vary greatly, depending on the thickness of the spread. The measurements of stained films have all been made on cover glass preparations in which the cells just touch but do not overlap. Films made on slides were used for the measurement of unstained preparations. Areas were selected where the cells were properly spread and did not overlap. The halos are much brighter on unstained films and so can be read more easily. In a stained

TABLE II
COMPARISON OF READINGS OF STAINED AND UNSTAINED FILMS OF NORMAL BLOOD

NO.	MEAN CELL VOL- UME BY HEMATOCRIT	MEAN CELL DIAMETER BY										CALCULATED MEAN CELL THICKNESS	VOLUME THICKNESS INDEX
		EAMONS ERIDMETER		PIJPET BLOOD-CELL- TESTER		BOCK ERYTHRO- CYTOMETER		CALCULA- TION FROM MEAN CELL VOLUME					
		STAINED MICRONS	UNSTAINED MICRONS	STAINED MICRONS	UNSTAINED MICRONS	STAINED MICRONS	UNSTAINED MICRONS	STAINED MICRONS	UNSTAINED MICRONS				
1	88	7.6	7.5	7.1	7.7	7.7	7.7	7.7	7.6	2.00	1.00		
2	91	7.8	7.6	7.3	7.9	7.7	7.9	7.6	7.7	2.00	0.97		
3	91	7.7	7.8	7.1	7.8	7.7	7.8	7.7	7.7	1.90	1.01		
4	85	7.3	7.3	7.2	7.5	7.6	7.5	7.3	7.6	2.00	1.11		
5	92	7.8	7.7	7.2	7.7	7.7	7.7	7.7	7.7	2.00	0.98		
6	84	7.6	7.6	7.2	7.8	7.8	7.8	7.7	7.5	1.85	0.96		
7	88	7.8	7.8	7.2	7.9	7.7	7.9	7.8	7.6	1.85	0.96		
8	82	7.6	7.5	6.9	7.6	7.7	7.6	7.7	7.5	1.90	0.95		
9	88	7.7	8.0	7.2	8.0	7.8	8.0	7.8	7.9	1.75	0.98		
10	91	7.8	7.8	7.1	7.8	7.7	7.8	7.7	7.7	1.90	0.99		
Average	88	7.7	7.7	7.2	7.8	7.7	7.8	7.7	7.6	1.90	0.99		

film the stain acts as a filter, hence, the colors of the halo are not just the same as in the unstained preparations. In every case, several measurements have been made and the average taken. Often measurements were made on more than one film. The mean cell thickness and the volume thickness index have been calculated as previously described.¹⁷

Table I shows the comparative measurements on the stained films only from ten normal persons. All films were made on cover glasses. The diameter as determined by the Pijper instrument is 0.5 mm less than with direct measurements and with the other instruments. I then compared the different diffraction methods on ten more normals (Table II), using both stained and unstained films. The average results with the different methods on unstained films are almost exactly the same. The results with the enometer and erythrocytometer also check closely with those found on stained films. The readings with the blood cell tester on stained films are again lower than on unstained films. These results show that a correction (about 0.5 mm) must be made for measurements on stained films when the Pijper instrument is employed.

In Table III are shown the comparative readings on stained films only with a group of patients showing a wide variation in mean diameter. The average diameter with the blood cell tester is again lower than with other methods, but when a correction is made, it checks closely with the other methods. The readings with the erythrocytometer are apt to be too low if the mean cell diameter is high, or too high if the mean cell diameter is low. In Table IV are tabulated the readings in a group of patients who had either a low volume index or a small cell diameter. All readings were made on unstained films. Here again the agreement is good except with the erythrocytometer, which does not give correct readings when the diameter varies widely from normal.

In Table V are collected the readings on unstained films when the mean cell volume is high as it characteristically is in pernicious anemia, or the cell diameter is high without a corresponding increase in volume, such as is seen frequently in liver disease. Here again, the readings with the Boek instrument are almost uniformly too low, and the average of 25 readings is below that with the other instruments.

In Table VI are shown the readings in a wide variety of conditions in which the volume index and mean cell diameter are within normal limits. Here there is a close check of the results obtained with the three instruments.

SUMMARY AND CONCLUSIONS

Diffraction methods are satisfactory for measuring the mean diameter of the erythrocyte.

In normal films or where the diameter varies little from normal, readings with Emmons' enometer, Pijper's blood cell tester, and Boek's erythrocytometer check closely with direct measurements.

Readings with the Emmons' enometer and Pijper's blood cell tester give accurate results even when the cells are very large or very small.

Readings with the Boek erythrocytometer tend to be too low if the cells are large and too high if the cells are quite small.

TABLE III
COMPARISON OF DIFFRACTOMETRIC WITH DIRECT MEASUREMENTS ON STAINED FILMS OF ABNORMAL MEAN CELL VOLUME

NO.	MEAN CELL DIAMETER BY						VOLUME THICKNESS INDEX	DIAGNOSIS
	MEAN CELL VOLUME BY HEMATOCHIT	DIRECT MEASUREMENT	EMMONS'S ERIDOMETER	PIPPER BLOOD-CELL-TESTER	BOCK ERYTHRO-CYTOMETER	CALCULATION FROM MEAN CELL VOLUME		
	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS		
1	63	7.0	7.1	6.6	7.5	6.8	1.60	Idiopathic hypochromic anemia
2	59	6.7	7.1	6.7	7.6	6.7	1.50	Idiopathic hypochromic anemia
3	59	7.0	7.0	6.5	7.3	6.7	1.55	Idiopathic hypochromic anemia
4	60	6.8	7.1	6.5	7.4	6.7	1.50	Idiopathic hypochromic anemia
5	68	7.2	7.3	6.9	7.4	7.0	1.60	Idiopathic hypochromic anemia
6	59	7.2	7.5	6.9	7.6	6.7	1.35	Lead poisoning
7	84	7.8	7.6	7.2	7.9	7.5	1.80	Idiopathic hypochromic anemia
8	84	7.7	7.5	7.0	7.5	7.5	1.90	Idiopathic hypochromic anemia
9	80	7.4	7.5	6.7	7.4	7.4	1.80	Idiopathic hypochromic anemia
10	90	7.7	7.6	7.1	7.5	7.7	2.00	Idiopathic hypochromic anemia
11	89	7.8	7.6	7.2	7.6	7.7	2.00	Idiopathic hypochromic anemia
12	144	8.9	8.8	8.4	8.4	9.0	2.40	Lead poisoning after treatment
13	130	8.8	9.2	8.8	8.4	8.7	2.00	Perniciou anemia
14	92	8.6	8.6	8.6	8.6	8.7	1.60	Perniciou anemia
15	93	8.2	8.4	8.1	8.5	7.7	1.70	Obstructive jaundice
16	105	7.9	8.3	7.9	8.2	8.1	1.90	Obstructive jaundice
17	97	7.7	7.7	7.2	7.6	7.9	1.08	Hemolytic anemia
18	86	7.2	7.3	6.9	7.4	7.6	1.13	Spherocytic jaundice
19	90	6.2	6.3	5.9	6.6	7.7	1.82	Spherocytic jaundice
20	160	6.4	6.5	5.9	6.6	9.3	4.80	Spherocytic jaundice
21	77	6.4	6.4	5.9	6.6	7.3	2.67	Spherocytic jaundice
22	78	6.5	6.6	6.2	6.9	7.4	1.35	Spherocytic jaundice
23	110	6.5	6.4	6.2	6.8	8.2	1.24	Spherocytic jaundice
24	87	6.6	6.4	6.1	6.6	7.7	1.93	Spherocytic jaundice
25	87	6.9	6.6	6.4	6.7	7.7	1.53	Spherocytic jaundice
Average		7.4	7.4	7.0	7.5	7.7	2.55	Spherocytic jaundice

TABLE IV
COMPARISON OF DIFFRACTION METRIC MEASUREMENTS WITH DIFFERENT INSTRUMENTS ON UNSTAINED FILMS OF BLOOD WITH DECREASED MEAN CELL VOLUME

NO	MEAN CELL DIAMETER BY						DIAGNOSIS
	MEAN CELL VOLUME BY HEMATOCYT	EMMONS EPHEMETER	11PPE BLOOD CELL TESTER	ROCK ERYTHRO CYTOMETER	CALCULATION FROM MEAN CELL VOLUME	CALCULATED MEAN CELL THICKNESS MICRONS	
	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	VOLUME THICKNESS INDEX	
1	85	71	71	76	75	116	Chronic pharyngitis
2	77	67	67	69	71	131	Spherocytic anemia
3	78	77	77	76	77	131	Idiopathic hypochromic anemia
4	86	72	73	76	75	117	Hypothyroidism
5	85	71	71	74	75	116	Essential thrombopenia
6	81	77	76	78	63	130	Banti's disease
7	77	74	73	74	73	180	Scurvy of femur
8	77	77	77	79	73	165	Idiopathic hypochromic anemia
9	82	75	75	76	74	185	Nutritional deficiency disease
10	61	78	77	78	63	130	Idiopathic hypochromic anemia
11	92	77	76	78	63	130	Banti's disease with hemorrhage
12	72	77	76	78	71	180	Pemphigus
13	58	81	80	80	67	115	Idiopathic hypochromic anemia
14	75	77	78	76	73	185	Hemorrhage from duodenum ulcer
15	68	78	76	77	70	165	Idiopathic hypochromic anemia
16	56	75	71	78	66	130	Idiopathic hypochromic anemia
17	73	64	67	71	73	225	Spherocytic anemia
18	75	77	76	76	73	145	Hemorrhage from duodenum ulcer
19	65	73	73	74	73	200	Idiopathic hypochromic anemia
20	89	73	73	74	69	160	Chronic toxicities
21	87	73	73	75	76	215	Idiopathic hypochromic anemia
22	69	76	75	76	75	112	Idiopathic hypochromic anemia
23	65	80	80	78	70	185	Peptic ulcer
24	63	81	82	80	68	130	Idiopathic hypochromic anemia
25	64	78	77	78	69	120	Chronic hemorrhage
Average		75	75	76		115	

TABLE V

COMPARISON OF DIFFRACTOMETRIC MEASUREMENTS WITH DIFFERENT INSTRUMENTS ON UNSTAINED FILMS OF BLOODS WITH INCREASED MEAN CELL VOLUME OR INCREASED MEAN CELL DIAMETER

NO.	MEAN CELL DIAMETER BY					DIAGNOSIS
	MEAN CELL VOLUME BY HEMATOCRIT	EMMONS ERIDROMETER	PIPPER BLOOD-CELL-TESTER	BOCK ERYTHRO-CYTOMETER	CALCULATION FROM MEAN CELL VOLUME	
	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	
1	103	7.7	7.8	6.6	8.0	Hemolytic jaundice
2	128	8.8	9.0	8.4	8.7	Pernicious anemia
3	95	8.2	8.3	7.9	7.8	Nutritional deficiency disease
4	96	7.4	7.6	7.4	7.8	Pernicious anemia under treatment
5	96	8.7	8.8	8.4	7.8	Cirrhosis of liver
6	124	9.5	9.4	8.9	8.6	Pernicious anemia
7	122	8.6	8.6	8.1	8.6	Hemolytic anemia
8	103	8.4	8.4	8.1	8.0	Pernicious anemia ?
9	83	8.3	8.4	8.1	7.5	Carcinoma of liver
10	99	8.0	8.0	7.9	8.0	Herpes zoster
11	108	7.9	8.1	8.0	8.2	Pernicious anemia under treatment
12	81	8.9	8.8	8.4	7.4	Cirrhosis of liver
13	96	8.6	8.7	8.4	7.9	Cirrhosis of liver
14	84	8.0	8.0	7.8	7.5	Pernicious anemia under treatment
15	104	8.4	8.5	8.3	8.1	Pernicious anemia under treatment
16	92	8.9	9.0	8.6	7.8	Cirrhosis of liver
17	94	8.3	8.4	8.1	7.8	Cirrhosis of liver
18	97	7.7	7.7	7.9	7.9	Pernicious anemia under treatment
19	100	7.9	7.9	8.0	8.0	Pernicious anemia under treatment
20	96	8.1	8.2	8.0	7.9	Pernicious anemia ?
21	113	9.1	9.2	8.7	8.3	Pernicious anemia
22	100	8.3	8.4	8.4	8.0	Pernicious anemia
23	120	8.9	9.0	8.6	8.5	Pernicious anemia
24	119	9.0	9.2	8.8	8.5	Hemolytic jaundice
25	96	7.9	8.1	7.9	7.9	Pernicious anemia
Average		8.4	8.5	8.2		

TABLE VI
COMPARISON OF DIFRACTOMETRIC MEASUREMENTS WITH DIFFERENT INSTRUMENTS ON UNSTAINED BLOOD FILMS OF ANEMIAS WITH
NORMAL MEAN CELL VOLUME

NO	MEAN CELL VOLUME BY HEMATOCRIT	MEAN CELL DIAMETER BY					CALCULATED MEAN CELL THICKNESS	VOLUME THICKNESS INDEX	DIAGNOSIS
		ELMONS EROMETER	PIJPER BLOOD CELL TESTER	BOCK ERYTHRO CYTOMETER	CALCULA- TION FROM MEAN CELL VOLUME				
					MICRONS	MICRONS			
1	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	1.04	Pernicious anemia in remission
2	90	76	75	76	77	77	200	1.08	Osteomyelitis
3	90	75	76	75	77	77	205	1.00	Chronic bronchitis
4	80	76	77	77	77	76	190	0.93	Progressive central muscular atrophy
5	84	77	77	77	77	75	180	0.95	Dyspituitarism
6	85	77	77	77	77	76	180	1.09	Bronchial asthma
7	91	75	77	77	77	77	205	0.97	Cystitis
8	88	77	77	77	77	75	190	0.90	Pernicious anemia in remission
9	84	78	77	79	75	75	180	0.93	Pernicious anemia in remission
10	84	77	79	78	75	77	180	1.01	Nutritional deficiency disease
11	01	77	77	78	77	77	195	1.01	Nutritional deficiency disease
12	81	76	77	77	74	74	180	0.95	Lead poisoning
13	83	77	78	77	76	77	180	1.22	Obstructive jaundice
14	91	73	76	75	75	77	220	1.04	Peripheral neuritis
15	83	74	73	75	75	75	195	1.02	Myxedema
16	81	74	75	75	74	76	190	0.95	Spastic colon
17	85	77	77	78	76	76	180	1.06	Gallstones
18	85	74	75	75	76	76	200	1.07	Pernicious anemia in remission
19	84	74	76	74	76	75	200	0.98	Pernicious anemia in remission
20	87	75	77	75	75	76	190	0.90	Pernicious anemia in remission
21	84	79	79	78	76	75	175	0.90	Nutritional deficiency disease
22	84	78	75	77	75	77	175	1.05	Pernicious anemia in remission
23	95	77	77	77	78	76	205	0.92	Diabetes mellitus
24	80	74	75	75	76	75	200	0.94	Hypertension
25	84	78	77	77	75	76	175	0.97	Hypometabolism
25	88	77	78	76	76	76	190		
Average		76	76	76	76	76			

The Emmons' eriometer has the advantage of portability and not requiring a dark room.

The Pijper blood-cell-tester is excellent for comparing an abnormal with a normal film and also indicates the degree of anisocytosis as well as the mean diameter.

The readings are the same with stained as unstained films with the Emmons and Boek instruments.

With the Pijper instrument, the diameter measures about 0.5 mm. less on a stained than on an unstained film.

For direct measurements, a film projected on the ground glass of a photomicrographic camera is very convenient. The flar micrometer is the most satisfactory ocular micrometer.

The calculation of the diameter from the mean volume by Warburg's nomogram is correct only when the variation from normal affects diameter and thickness equally.

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ERYTHROCYTE SEDIMENTATION AND ANEMIA

A PRELIMINARY REPORT

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IT IS the purpose of this communication to present and attempt to substantiate by clinical evidence the claim that the relative number of circulating erythrocytes in the blood plasma has very little, if any, influence per se on the *speed of settling* of the red cells. It is further claimed that any effect that may exist is so varying in degree within the same individual, and differs so greatly among different individuals, that any attempt at 'correction' of the sedimentation rate in a secondarily anemic patient in large part obscures the true nature of the curve of settling, and thus reduces the value of the reading by introducing an extraneous and above all, an essentially inconstant factor.

The term "secondary anemia" is stressed, as reference is made only to a diminution in the relative number of circulating red cells as caused, for example, by hemorrhages or by peripheral destruction of normally formed cells as in malaria. Diseases of the blood forming organs, such as the leucemias and pernicious anemia, are considered in this connection not as forms of anemia proper, but as disease entities having a marked effect on the sedimentation rate independent of the diminution in the number of circulating red cells. For the purpose of substantiating the claims herein put forth, the most clear cut example would be a loss of blood without hemorrhage into peroral body cavities or tissues, such as might occur in a penetrating gastric ulcer, for the following reasons: a gastric ulcer does not, as a rule, have a noticeable effect on the sedimentation rate in itself, so that hemorrhage from this source represents a loss of blood pure and simple, such as may be encountered in actual clinical experience. Reference is made to interstitial hemorrhage and other extracirculatory accumulations of blood within the body (exclusive of the digestive tract), as these factors are likely to affect the sedimentation rate as a result of the tissue reaction and absorption of abbaun products.

An example of the dovetailing of various factors, on the other hand, is to be found in cases of anemia accompanying therapeutic malaria in general paresis in which the syphilitic agent, the malarial agent, the hyperthermia per se, and possible complicating factors such as decubitus ulcers, etc., all play a part in altering the sedimentation rate, regardless of whether there is an accompanying anemia or not (which, in turn, may be the direct result of either the paresis, or the malaria, or both).

The above examples represent opposite extremes, indicative of the type of material and the pitfalls encountered by the worker in this field. The sedimentation readings herein presented in fragmentary form were obtained for the greater part, but not exclusively, from patients inoculated with double tertian

malaria. All cases were, however, well worked up clinically and followed after termination of the malarial treatment, and did not show, as far as observed, any of the few conditions known definitely to *decrease* the rate of settling.^{1,2} Moreover, although roughly representing a specific clinical group, the individual cases have contributed every known type of erythrocyte sedimentation curve, and it was this very observation which first called my attention to the subject matter herein discussed.

In view of the great number of factors influencing the erythrocyte sedimentation rate, as well as the admitted fact that the actual causes for changes in the

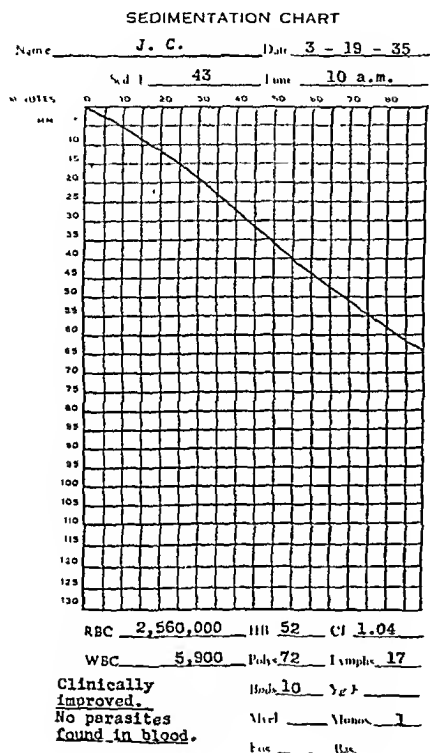


Fig. 1.

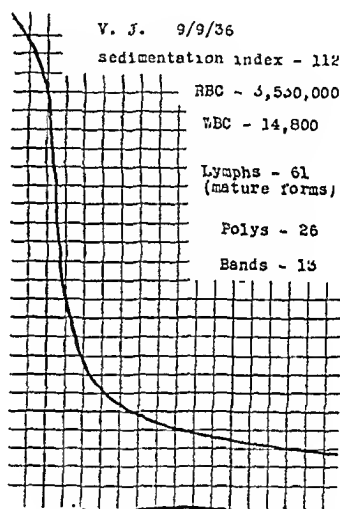


Fig. 2.—V. J., an example of a markedly pathologic curve accompanying a moderately low red cell count in a case of lymphatic leucemia, "aleucemic" form. This patient died a few days after the above reading was obtained.

rate are still at best only in part determined, it would seem that any attempt to eliminate completely and routinely one of the supposed factors, even without a clear knowledge of the exact manner in which the latter might exert an influence, is perhaps premature and certainly represents a tampering with a physiologic phenomenon which in its "unmodified" form is quite capable of diagnostic interpretation in the hands of the experienced observer.³ The term "unmodified" is purposely put in quotation marks, as I am aware of the fact that, with the usual technique, the test cannot be considered unmodified in the strictest sense of the word. It is, in the first place, a test *in vitro*, and, second, the blood employed contains an anticoagulant. Furthermore, the average laboratory procedure involves considerable contact with air, and the element of elapsing time between venipuncture and setting up of the blood,^{4,5} although relatively negligible within a more or less definite limit, is rarely given sufficient attention to

render it a constant factor, even when a group of sedimentation rates is read simultaneously. We may, however, disregard at least the factors of the glass container and anticoagulant,⁸ as they are present in every instance, and, at least within the limits of the particular technique used (e.g., Linzenmeier, Westergren, Cutler, etc.), remain constant, except for gross errors introduced by faulty manipulation. As a matter of fact, various attempts at correlation have shown that there is no great element of discrepancy involved even in comparing results with the various classical techniques.⁷

At this point it seems appropriate to diverge, in order to review briefly as a group all factors that might have an effect on the blood *in vitro*, and that have been considered for that reason by various authors. In addition to the form of the glass vessels, the kind of anticoagulant employed, and the time interval between obtaining the blood and setting up the test, factors to which reference has

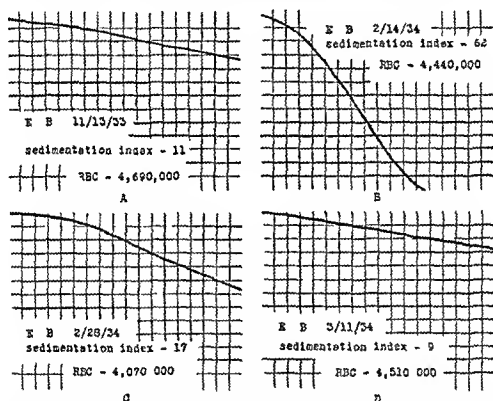


Fig. 3—L. B. an example of the most frequently encountered finding (i.e. a temporary marked drop of the sedimentation curve with a relatively stable red cell count (acute gangrenous appendicitis operation and uneventful recovery in an otherwise healthy nineteen year old nurse)).

already been made, the following have received attention at the hands of a number of workers: the temperature at which readings are made,^{4, 8, 9} the meteorologic conditions,¹⁰ the position of the glass container,⁸ etc. It is not within the scope of this report to discuss this aspect of erythrocyte sedimentation in detail, but it may be said at this point that of all the above named factors, although some of them have been found definitely to affect the rate of settling, none need be considered seriously within the limits of so called "normal" working conditions beyond the point of avoiding extreme fluctuations in temperature (i.e., maintaining average room temperature) and unnecessary delay in starting the test: using the same anticoagulant consistently, setting up the tubes as prescribed for the technique employed, and keeping all glassware properly clean. In other words, it is necessary only to observe the precautions customary in a well conducted laboratory. This statement would seem to be an invitation to inaccuracy,

but the attitude it represents is based on the most outstanding characteristic of the sedimentation phenomenon, which in turn furnished the basic motive for the presentation of this report.

It seems to the writer that there exists a tendency to lose sight of the fact that the rate of settling of the red blood cells is *essentially a nonspecific biologic phenomenon with only approximate values*, or is so at least as far as our present knowledge goes. It is, for this reason, impossible to draw a sharp line of demarcation between what represents a "normal" and what a "pathologic" reading, as witness the confusingly wide range of so-called "normal limits," as given by different authors.^{1, 11-15}

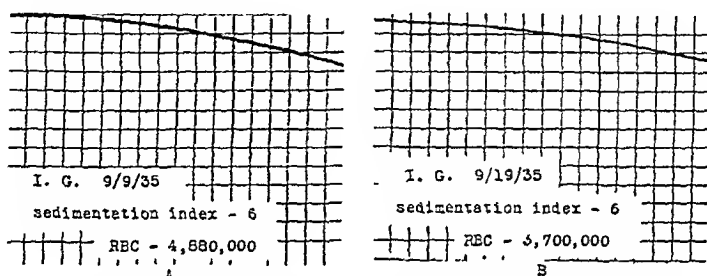


Fig. 1.—I. G., an example of an appreciable drop in the red cell count with an unchanging normal curve, interpreted as "prognosis favorable," in a paretic inoculated with double tertian malaria. Patient's subsequent course satisfactory.

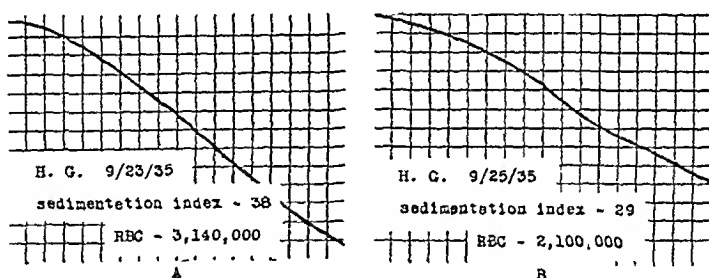


Fig. 5.—H. G., an example of marked and progressive anemia with an only moderately pathologic and improving curve, interpreted as "prognosis favorable," in a paretic with double tertian malaria. Patient's subsequent course satisfactory.

It is in great part this very absence of specificity and of definite normals that has led to the great variety of approaches in an attempt to determine the agents responsible for the fluctuations in the rate of settling. The literature today abounds in references to such widely divergent etiologic factors as: the time of day at which the blood is obtained, the degree of venous stasis present at the time of venipuncture,¹⁶ the effect of meals,¹⁷ endocrine imbalance (e.g., thyrotoxicosis, epinephrine content of the blood, diabetes mellitus,¹⁸ etc.), variations in amount and proportion of the various protein components of the blood plasma (albumin, globulin, fibrinogen, etc.),^{19, 20-23} variations in the electric charge of the erythrocytes,¹ variations in lecithin and cholesterol content, as well as of other lipoids, and hyperthermia, irrespective of its cause, to list only a few of the factors of a general character representing pathologic conditions or extreme physiologic fluctuations. In the final analysis, it is possible to correlate with a fair degree of accuracy the degree of increased rate of settling of erythro-

cytes with the amount of cell destruction going on in the body, but this working theory is, after all, only provisional and based on observation rather than complete understanding

When the problem is approached from the point of view of specific disease entities and their effect on the rate of settling, another grouping is obtained, best represented at present, although necessarily by no means exhaustive in its scope, by the compilation of Cutler¹⁴ In this compilation, diseases are grouped according to whether they are "accompanied by an abnormal sedimentation rate," whether they "influence the sedimentation rate very little if at all," and as "not influencing the sedimentation rate" There is, unfortunately, no mention of anemias in this list It may, however, be stated briefly here that pernicious anemia and the various leucemias are admitted to have a very definite and constant effect on the sedimentation rate, which is quite distinct from any effect that might be caused by an uncomplicated secondary anemia (see Fig 2)

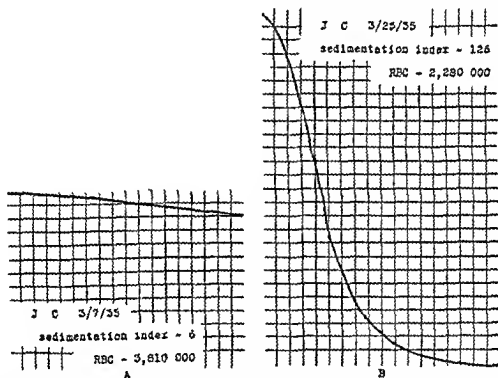


Fig. 6—J C an example of marked and progressive anemia with a sudden change from a normal to an extremely pathologic curve the latter pronounced even in proportion to the anemia interpreted as prognosis grave in a parietic with double tertian malaria Patient died within twenty four hours of obtaining the above reading

The material herein referred to consists exclusively of sedimentation curves charted graphically, rather than of one time readings It is my opinion that, despite indefinite normals, a curve is capable of direct interpretation on the basis of consideration of several factors, such as initial speed of settling, fluctuations in the speed throughout the period of reading, the one hour "index," the final reading after settling is complete and, last but by no means least, a comparison of repeated tests on the same individual^{4, 21}

Just how much importance may be ascribed to any one or all of these factors is entirely a matter of "habitation" on the part of the physician, that is to say, of the amount of experience acquired by the routine reading of sedimentation curves in conjunction with a consideration of red cell count, white cell count, differential, urinalysis, temperature chart and other laboratory and clinical data The chart used at Springfield State Hospital, Maryland, during

the years 1934, 1935, and 1936, when the material herein referred to was obtained, was designed by the writer with the above principles in mind (Fig. 1). On this chart were recorded the readings obtained with the Westergren tube, using citrate as an anticoagulant.⁶

In the attempt to augment the utility of the sedimentation phenomenon as a laboratory test, a great many modifications of the original methods have been evolved, but the most conspicuous of these is the so-called "correction" of the rate, intended to compensate for a low red cell count either by subtracting from the reading a number of millimeters in each case supposedly equivalent to a certain volume or number of red cells, or by changing the plasma-cell ratio in vitro before reading.²⁵⁻³⁰ These corrections do not, of course, take into account the *type of curve* obtained. In its extreme form, this procedure has led to the adoption of "correction charts." Only the corrected reading thus obtained is referred to for diagnostic interpretation in these modifications. The considera-

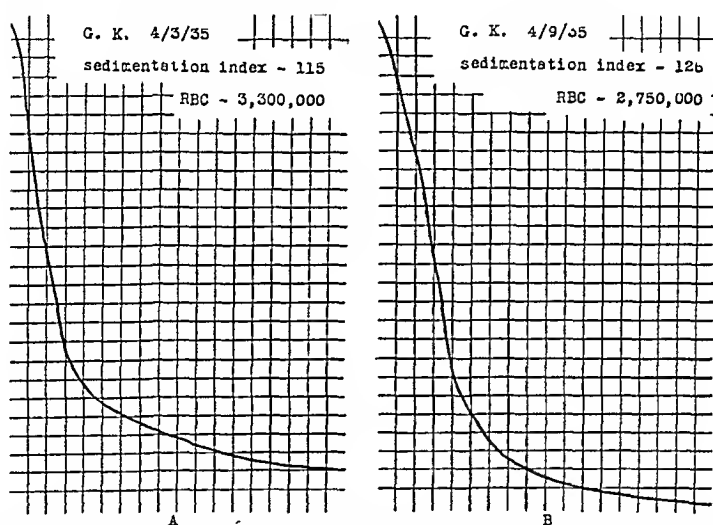


Fig. 7.—G. K., an example of progressive, but less pronounced, anemia with an extremely pathologic curve from the outset, interpreted as "prognosis hopeless," in a paretic with double tertian malaria. Patient died within twenty-four hours of obtaining the above reading.

tion of these methods does not, of course, include correction for the factor of cell volume shrinkage produced by the particular anticoagulant employed.³⁰

In the present report, preparatory to demonstration of material, it is maintained that the effect of a moderate (and in some cases a marked) diminution in the number of circulating red cells in the blood has a negligible effect on the sedimentation curve, and that such effect as may be present impairs the diagnostic value of the test far less than the essentially uncertain and even less well understood effect produced by "correcting" the reading. Tests conducted with accurately measured numbers of red cells increased and decreased in vitro,²⁹ have, to be sure, demonstrated that the sedimentation rate increases apparently in proportion to the number of cells removed from the plasma. Such consistency has *not*, however, been determined to date in blood tested as obtained from the circulation without further manipulation. In other words, attempts at correcting the rate are, at least in the present state of our knowledge, advised

against by the writer as introducing a misleading element of pseudoaccuracy into an essentially inaccurate test. It is contended that the following samples of case material substantiate these claims. The laboratory tests involved were carried out in the course of routine work, with occasional checks and rechecks, and the examples illustrated are purposely chosen as offering sufficiently marked variations from the normal to provide a margin for minor technical errors.

I am aware of the fact that the red cell count in itself is an only approximate indicator of the *volume relationship* between plasma and cells, as well as of the fact that, with a diminished total cell volume, the *final reading* will be quite different from that of normal blood. The statements made in this report have reference exclusively to the *speed of settling*, not to the distance traversed by the red cells before packing occurs. In most of the cases included in this report, twenty-four-hour readings were made, and in some of the earlier cases, packed cell volume determination, but neither item was recorded in every instance, as this material (see above) was not accumulated in the course of experimental work. No figures of this kind are therefore given here. Moreover, attention was paid in every instance to the general appearance of the erythrocytes, both in regard to size and uniformity of shape, in the course of the red cell counts and smear studies, and the graphs chosen for illustration of this report were picked from the standpoint of representing sufficiently wide variations to take into account the factor of fluctuating total cell volume as separate from the cell count.

No attempt will be made within the confines of this report to give complete case findings, or to reproduce the complete series of sedimentation rates for any one individual.

SUMMARY

1. It is contended that the relative number of erythrocytes in the plasma has a negligible effect per se on the rate of settling of the red cells, and that such effect as may exist is inconstant.

2. A number of intrinsic and extrinsic factors that have received attention as possibly affecting the rate of erythrocyte settling is listed, as indicative of the present status of sedimentation as a nonspecific and only partly understood phenomenon.

3. It is maintained that attempts at "correction" of the sedimentation rate by compensating for anemia, and especially the use of "conversion charts," represent pseudoaccuracy and in large part invalidate the readings.

4. It is further maintained that the "unmodified" sedimentation curve, graphically recorded, is a valuable diagnostic aid when correlated with other laboratory and clinical data.

5. A chart is shown for the joint recording of sedimentation curve, sedimentation index, red and white cell count, differential count, and special information.

6. A number of selected curves is reproduced in substantiation of the contention that the sedimentation rate and the red cell count may fluctuate independently of one another, and even move in opposite directions in the same individual, in the course of a pathologic process.

7. If an individual with a low red count, but otherwise essentially normal findings, shows a definitely pathologic sedimentation curve, that individual's true ailment has not been determined and is of a serious nature.

Since writing the above, two articles have come to my attention, one of which appeared in the May, 1937, issue of the *J. LAB. & CLIN. MED.*, the other in the October 16, 1937, issue of the *J. A. M. A.* The former was entitled "The Sedimentation Rate in Experimental Anemia (Rabbit)," by R. O. Gregg, and the latter "The Erythrocyte Sedimentation Rate," by E. G. Bannick, R. O. Gregg, and C. M. Guernsey, of Rochester, Minn.

The attitude of the authors, as outlined in their joint paper, coincides so perfectly with my own on this subject that I feel mention should be made of their work in this connection. The experiments carried out on rabbits represent, in my opinion, a great step forward in the refutation of the conclusions arrived at by *in vitro* methods, and in the elimination of too complicated techniques evolved chiefly as a result of such methods and conclusions.

Despite the outlook for eventual general agreement on the factor of anemia in the interpretation of the sedimentation rate, there remain individual differences of opinion on the relative value of graphic curves or one-time readings. This latter difference would appear to be still a matter largely of personal experience, and should not retard the eventually general acceptance of erythrocyte sedimentation readings as a valuable aid to diagnosis and treatment.

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‘INTEGRATIVE ACTION’ OF MIXTURES OF SIMPLE ORGANIC SUBSTANCES IN OXIDATION REDUCTION PHENOMENA PRODUCED BY AUTOCLAVING*

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THIS paper considers the persistence of reduction of methylene blue in mixtures of certain organic acids and sugars at pH 5.6 and 8.0 after autoclaving at 15 pounds pressure for twenty minutes. Such persistence is compared with that of cysteine hydrochloride (Eastman) solutions of varying pH and concentrations, control solutions, and D-glutamic acid cysteine HCl mixtures. “Integrative action” is considered as of three types.

All solutions contained 0.001 per cent methylene blue. The stock solution of methylene blue was made up to 0.1 per cent with distilled water of pH 5.6. Observations and tests were made under aerobic conditions. The solutions were kept at room temperature in diffused light. Solutions were contained in 2 by 17 cm cotton plugged test tubes. The pH was adjusted colorimetrically before and after autoclaving on solutions to which methylene blue had not been added.

When reduction was persistent it was recorded by the Hardy spectrophotometer¹ at intervals of six to forty eight hours. The solutions were poured gently into spectrophotometric cells of 1 cm inner thickness and gently poured back into the test tubes to be reobserved at a later time in a similar manner. The terms complete oxidation and complete reduction as used in this paper signify an oxidation and reduction as complete as recordable by the eye or the spectrophotometer.

The term “integrative action” has been used in describing results since certain mixtures show more persistent reduction than either substance separately.

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¹Methylene blue U S P Chemical C I 922 total dye content 38 per cent National Aniline & Chemical Co Inc New York

Therefore, neither substance can be said to catalyze a change in the other or in the methylene blue. Apparently the physical force of autoclaving activates the change.

It frequently has been observed that after autoclaving certain bacteriologic mediums containing methylene blue show temporary reduction to the eye. The factors involved are indeterminable because of complexity of the medium. They probably are of importance since many recent reports indicate that freshly autoclaved medium is best for growth of certain organisms and that reduced potential insures a more secure foothold for better organismal growth.

Ease of oxidation of reducing sugars and transportation of hydrogen by various agencies to methylene blue are well known. Wurmser and Gelso² report a substance they call G' which is developed in alkaline glucose solution protected from O₂ and which is capable of rapid oxidation in the cold by dehydrogenation in the presence of methylene blue or in the presence of molecular oxygen. They consider that probably it is a di-enolic form of glucose and that in the cell there exists an oxidation-reduction system similar to that of the glucose solution. It is possible that a similar factor is important in the reductions I have noted in mixtures containing sugars. Palit³ and Palit and Dhar⁴ have noted retardation of oxidation in the presence of fats or nitrogenous substances. This observation may be correlated with those to be described since successive reductions followed by delayed oxidation would result in a composite picture of more persistent reduction.

The solutions studied are listed on the opposite page. A control of each substance of the weight used in the mixture was run. In addition, three times the weight used in the mixture of two characteristic integrating substances (glycine and dextrose) were run as controls. The results observed for these corresponded closely with those noted for other controls. It is realized that contaminants of various metal salts were present. It is doubtful that these varied our results since they were uniformly present and many illustrative solutions were tested. All solutions were made up at both pH 5.6 and 8.0 (exception Nos. 13, 14, and 15 which were pH 5.0 and 8.0). They usually were run in triplicate and were rechecked by reautoclaving. With the exception of cysteine HCl solutions, where oxidation had precipitated the insoluble cysteine, results showed striking correspondence.

With the exception of cysteine solutions (Nos. 13, 14, and 15), because of rapid oxidation of the methylene blue, tubes were observed only with the eye. At both H-ion reactions the mixture of d-glutamic acid and succinic acid acted like the controls.

At pH 5.6 (Nos. 1 through 12) the controls showed little if any reduction of methylene blue. Mixtures with the exception of No. 5 showed complete reduction to the leucoform on removal from the autoclave followed by rapid complete oxidation in five to ten minutes.

At pH 8.0 (Nos. 1 through 12) controls and No. 5 showed none or very transitory reduction in no way to be confused with that of the mixtures. In the mixtures reduction was complete on removal from the autoclave to be followed by complete oxidation in one-half to two hours.

The factors which integrated with the sugars in the reduction apparently are not dependent on the amino group, since they are contained in succinic acid,

SOLUTIONS

- (1) 10 gm d glutamic acid, 10 gm dextrose
- (2) 10 gm d glutamic acid, 10 gm lactose
- (3) 10 gm d glutamic acid, 10 gm dextrin
- (4) 10 gm d glutamic acid, 10 gm sucrose
- (5) 10 gm d glutamic acid 0.6 gm succinic acid
- (6) 10 gm d glutamic acid, 0.6 gm succinic acid, 10 gm dextrose
- (7) 10 gm d glutamic acid, 0.82 gm succinic acid, 10 gm dextrose
- (8) 0.82 gm succinic acid, 10 gm dextrose
- (9) 0.79 gm malic acid, 10 gm dextrose
- (10) 0.79 gm fumaric acid, 10 gm dextrose
- (11) 0.52 gm glycine, 10 gm dextrose
- (12) 0.62 gm dl alanine, 10 gm dextrose
- (13) 0.25 gm cysteine HCl, 0.25 gm d glutamic acid
- (14) 0.125 gm cysteine HCl 0.125 gm d glutamic acid
- (15) 0.25 gm cysteine HCl 0.25 gm d glutamic acid, 0.25 gm dextrose

malic acid and fumaric acid. The factor peculiar to the sugars and apparently common to many sugars is present even in the "nonreducing sugar," sucrose, but to a lesser degree as indicated by persistence of reduction. Of the substances furnishing the other factors, glycine seems most active. The higher concentration of succinic acid seems to give a slightly more persistent reduction.

Various concentrations of cysteine HCl from 0.01563 gm per 100 cc to 10 gm per 100 cc of pH 5.0, 5.6 and 8.0 were studied. Results with 0.01563 per cent cysteine HCl corresponded to those of the mixtures discussed above. The reduction was more persistent in the more alkaline solution and less persistent in the acid solution. At a concentration of cysteine HCl intermediate between 0.125 and 0.25 per cent the relative length of time of persistence of reduction reversed, the acid solutions showing more persistent reduction. In acid solution ($\frac{1}{4}$ per cent cysteine HCl) there appeared in the first few hours an initial oxidation after complete reduction by the autoclave. This oxidation was followed by a second reduction which persisted about eight six hours.

In mixtures of d glutamic acid and cysteine HCl, (Nos. 13 and 14) reduction was prolonged about twelve to twenty-four hours over control cysteine solutions at pH 5.0 but not 8.0. This is considered a second type of integrative action different in three ways from that previously discussed, (a) G' factor cannot be postulated, (b) d glutamic acid increases length of reduction in a system already powerful, and (c) the action is in the more acid mixture. In the later instance in which there has been change with H ion concentration in the mixture showing integrative action sugar has not been used.

When a mixture of cysteine HCl, d glutamic acid and dextrose (No. 15, pH 5.0) were autoclaved changes considered a third type of integrative action occurred. The solutions vacillated between 10 and 80 per cent reduction of methylene blue over the six days of observation. Similar mixtures at pH 8.0 showed no decided difference from control cysteine HCl solutions.

It is accepted that a state of vacillatory oxidation reduction occurs in living cells. It is hoped that a study of the third type of integrative action will give us valuable information that can be applied to cell processes.

The expression persistence of reduction is used in preference to inhibition of oxidation since it is assumed that forces at play are preventing more rapid accomplishment of the more stable oxidized state. The reduced state, because of greater unstableness, seems more emblematic of the phenomena of life. Certain substances and conditions aid in maintaining the reduced state. Since drying

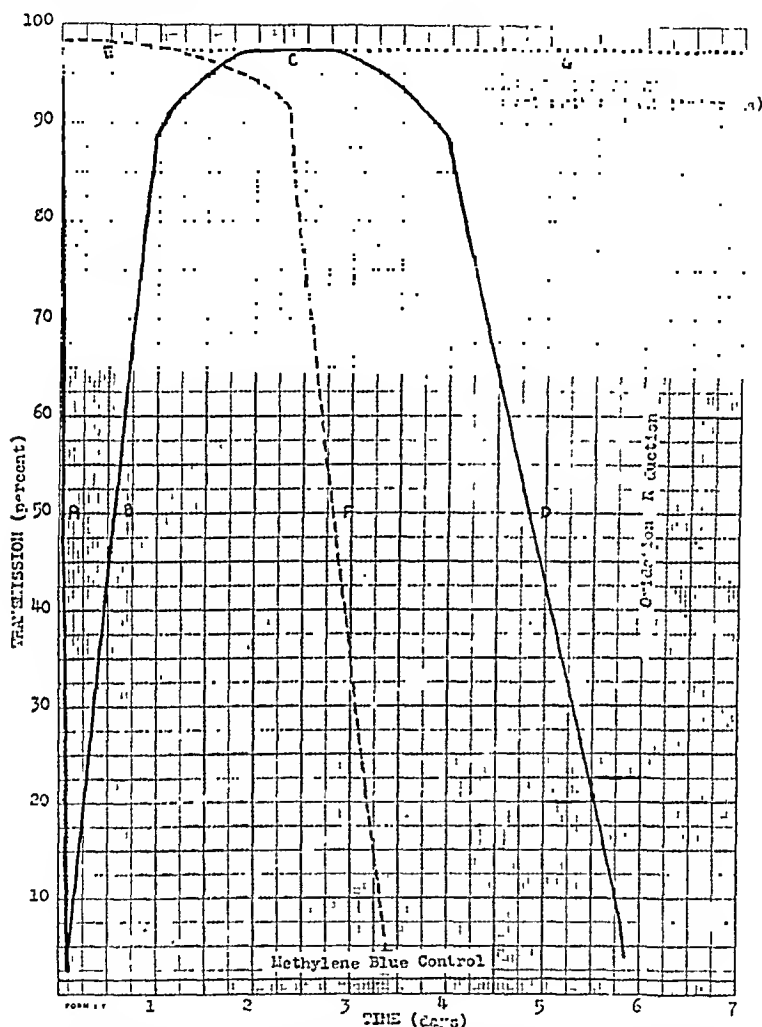


Chart 1.—The chart shows transmission curves for wave length 664 which has remained characteristic for 0.001 per cent methylene blue solution. The color variation was originally drawn by the Hardy spectrophotometer. On noting the percentages of transmission one notes the approximate percentage of reduced dye. The percentages must be corrected in accordance with the control methylene blue line in order to obtain more exact figures. It will be noted that neither complete oxidation nor complete reduction is attained.

Line A may be considered representative of what occurs when organic acids and sugars (pH 8.0) studied were autoclaved; there initially is noted 96 per cent reduced form which rapidly becomes oxidized so that only 1 per cent reduced form is charted.

Line A also appears in the more acid (pH 5.6) $\frac{1}{4}$ per cent cysteine solutions. It, however, again quickly reduces as seen in BC and is later oxidized (D). When glutamic acid is added to cysteine D may occur twelve to twenty-four hours later than in the control cysteine solution.

Curve EF is illustrative of 0.25 per cent cysteine at pH 8.0.

Line BCG is drawn to illustrate prolonged complete reduction (97 to 99 per cent) such as occurs in more acid (pH 5.0) concentrated (0.5 to 1 per cent) cysteine solutions.

The duplicate and triplicate curves studied were characteristic in type showing variations which seemed minor when one considers the variation probability.

and therefore concentration of reducing substances is one way of maintaining certain simple forms of life in a viable but inactive state, it may be presumed that when dilution of the cell's ingredients occurs (correct temperature aiding in establishing the ideal physicochemical state) sufficient oxidation takes place to establish the correct oxidation-reduction equilibrium for cellular growth.

The delay in oxidation noted when an amino acid was added to cysteine may be a phenomenon similar to that described by Barron and others* for ascorbic acid in which a heavy metal (copper) was the catalyst. It is probable, also, that in the systems studied, the rate of oxidation bears a relationship to the oxidation-reduction potential of the dye.

Activation of change by heat may create spatial relationships which help determine reduction of the dye. It is evident that more study is necessary on type two and three integrative action. Since autoclaving was a basic factor in these studies and used as a point of departure in observation, changes in solutions which had not been autoclaved were not studied.

SUMMARY

The object of this paper is to report "integrative action," subsequent to autoclaving, expressed in persistence of reduction of methylene blue. Such action is divided into three types:

a That of mixtures of organic acids and sugars which is more pronounced at pH 8.0 than pH 5.6

b That of mixtures of d-glutamic acid and cysteine HCl which is observable in solutions of pH 5.0 but not 8.0

c That of mixtures of d-glutamic acid, cysteine hydrochloride and glucose which shows a vacillatory state of partial reduction and partial oxidation in solutions of pH 5.0 but not 8.0

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A STIRRING DEVICE FOR USE IN MICROTITRATIONS*

PAUL LEVATINSKY, M.S., BOSTON, MASS.

THE use of an air current for stirring during microtitrations is an accepted procedure, but because of the lack of compressed air in some laboratories, or of proper reducing valves to control it, the method is not widely used. The device here illustrated is easily constructed and provides for adequate control of the air current.

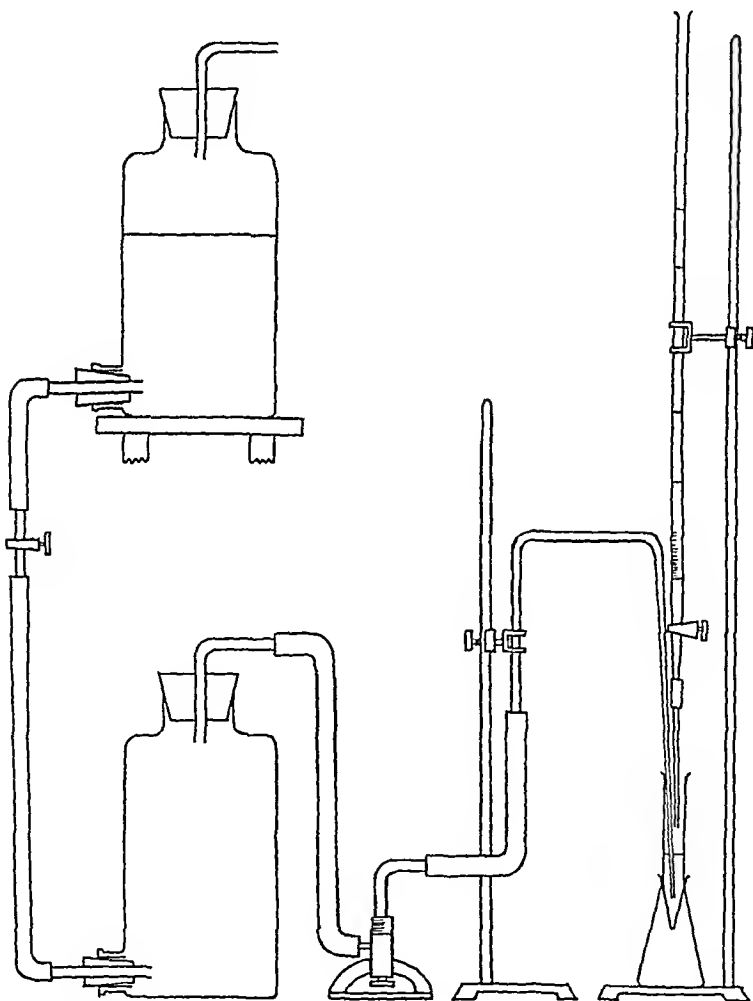


Fig. 1.

*From the Department of Biological Chemistry, Tufts College Medical School, Boston.
Received for publication, June 18, 1937.

The diagram is self-explanatory. Pressure is provided by water flowing from one aspirator bottle into another, thus displacing the air and forcing it first through the needle valve and then through the capillary tube which is immersed in the solution being titrated. When two 2 liter bottles are used, the supply of air will last for about an hour. After all the water has passed from one bottle into the other, the lower bottle may be disconnected and the positions of the two reversed. The needle valve is made from an old Tirill burner by removing the burner and soldering a short piece of copper tubing over the needle valve seat.

Since the volume of air used is so small, oxygen and carbon dioxide may be easily removed by connecting suitable absorption tubes in series, if either of these gases interfere with the titration.

A SIMPLIFIED SHAKING APPARATUS*

JOSEPH H. HOLT, RANGER, TEX.

IN SMALL laboratories where expense of equipment is an item, a suitable shaking apparatus for serologic tests is frequently a factor of major importance.

The shaking apparatus described in this article was designed to fill the need for a simple and yet efficient and inexpensive means of shaking test tubes.

The apparatus consists of a complete motor vibrator, such as is used by many beauty parlors and barber shops for massage. The motor is clamped to a ring stand by an adjustable clamp, or may be bolted to a wooden base. The end of the vibrator, to which the rubber tip is attached, has a small threaded hole. By means of a screw passed through the bottom, a small mailing carton or baking soda can is attached firmly to the end of the vibrator and the junction soldered. Washers were used to reduce the strain on the thin bottom of the carton. When in use, the tubes are placed in the carton, the lid screwed on, and the motor started. This imparts a vigorous shaking motion, the speed of which may be controlled by means of a small rheostat.

An apparatus such as described above has been used by the writer for many months, and the results have been entirely satisfactory in every respect.

*From the laboratories of West Texas Clinic and Hospital, Ranger.
Received for publication June 21, 1937.

A SIMPLE COLORIMETRIC METHOD FOR DETERMINATION OF ALCOHOL CONCENTRATION IN URINE AND BLOOD*

A. G. SHERTEL, M.D., LOS ANGELES, CALIF.

THERE is a definite need in legal and clinical medicine for a simple and accurate test for alcohol concentration in urine or in blood, a test which does not necessitate special distilling apparatus, and which may be applied anywhere, in places where elaborate laboratory facilities are not available.

Many alcohol tests have been described during the last few years, but they all have certain limitations, inasmuch as they lack simplicity, or require elaborate technique and apparatus.

Most of the methods are based on the oxidation of ethyl alcohol by potassium dichromate. The alcohol is either distilled from the specimen as in the method of Heise,¹ or absorbed by a current of purified air passing through the reagent (method of Bogen,²) or the alcohol is evaporated and absorbed by sulphuric acid in a hermetically closed flask (method of Widmark³).

Of the methods now available, the Widmark method is the most convenient, as it does not require distilling or aerating apparatus. It does require, however, a special S-shaped tube for absorption of the blood. This test calls for very small quantities of blood, but it is a titration method and necessitates skillful and elaborate technique. Abels⁴ modified the Widmark method by transforming it into a colorimetric one. He also substituted filter paper for the S-shaped tube. As in the Heise and the Bogen methods, the partially decolorized potassium dichromate is compared with a series of standards, usually 15, each corresponding to a definite percentage of alcohol. As the standards are not stable, it is necessary each time to prepare a series of fresh standards, which makes these methods very tedious.

In the method herein described, the blood or urine specimen to be tested is evaporated in a tightly sealed flask, and the alcohol oxidized by potassium dichromate. The partially decolorized potassium dichromate is compared in a colorimeter with the potassium dichromate reagent. A dark blue filter is used to facilitate matching colors, as the partially decolorized potassium dichromate has a blue greenish tint, due to the presence of chromic sulphate.

As 1 c.c. of the potassium dichromate reagent corresponds to 1 mg. of alcohol, the percentage of alcohol in the specimen is determined from the difference in potassium dichromate contents between the reagent standard and the partially decolorized reagent. The chromic sulphate formed by oxidation of alcohol absorbs a certain amount of light in the colorimeter. This has been taken into consideration by diluting the standard and the unknown accordingly.

APPARATUS AND REAGENT REQUIRED

Fifty cubic centimeter Pyrex Erlenmeyer flask.

One rubber cork in which a monel or nichrome wire clip is inserted to keep the filter paper in place.

*From the Department of Pathology of the College of Medical Evangelists and the Laboratories of Los Angeles County Hospital, Los Angeles, Calif.

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Filter paper strips of medium thickness, $1\frac{1}{2}$ by 2 inches.

Special blue filter.*

Accurate 2/10 c.c. pipette.

REAGENT

Solution of 0.4262 per cent of potassium dichromate in 50 per cent (by volume). Sulphuric acid (1 c.c. of this reagent corresponds to 1 mg. of alcohol.)

PROCEDURE

Roll one filter paper strip into a small cylinder and insert it into the clip attached to the cork. Introduce into the Erlenmeyer flask 1 c.c. of reagent. Absorb into the filter paper 2/10 c.c. of urine or blood to be examined.



Fig. 1.

The blood may be oxalated or freshly drawn from the ear lobe or the tip of the finger. Insert the cork immediately, but not tightly into the Erlenmeyer flask. Heat the neck of the flask from all sides over a Bunsen burner, and then press the cork very tightly into the neck of the flask. Place in a one liter beaker a small quantity of water, and into this put the flask. Adjust the volume of water in the beaker so that the flask does not float. Cover the beaker with a Petri dish or a watch glass. Boil the water in the beaker gently for fifteen minutes. Remove the flask from the beaker. Remove the cork gently from the flask, cool lower portion under tap, and add 4.3 c.c. of distilled water. Into a clean test tube introduce 2.5 c.c. of reagent and add to it 10 c.c. of distilled water. It will be seen from this that whereas 1 c.c. of reagent standard is diluted to 5 c.c., 1 c.c. of unknown is diluted to 5.4 c.c. Approximately 1/10 c.c. of the blood remains on the filter paper as unevaporated material.⁴ The difference in the dilution is the necessary correction for absorption of light in the colorimeter by chromic sulphate. Compare in any microcolorimeter, using the blue filter and setting the unknown at 10. Calculate from the following formula:

$$\left(1 - \frac{R}{10}\right) \times 500 = \text{mg. Alcohol per 100 c.c.}$$

R = Reading of the reagent-standard.

For example: R is 7.4; then $\left(1 - \frac{7.4}{10}\right) \times 500 = 130$ mg. alcohol per 100 c.c.

*Lantern blue glass filter No. 554, manufactured by MacGregor Instrument Co. of Needham, Mass.

COMPARATIVE VALUE OF THE HINTON TEST

In the present or third group of 3,000 patients, we found 427 serologically positive individuals. Of these, 399 gave a positive Hinton reaction. The test giving the closest number of positives was again the Kahn with 359. Fifty-one patients were negative to all other serological tests except the Hinton. Of these, 7 cases remained unconfirmed and are classified as false positives. All the other tests combined produced 9 unconfirmed cases.

The Hinton reaction failed in 4 cases which were positive to one or more of the other tests.

SUMMARY OF THE THREE STUDIES

In 9,000 patients, 1,704 patients gave a positive reaction to one or more of the four laboratory procedures used; 37 gave a doubtful result, and 7,259 were entirely negative.

Of the 1,704 positive cases the Wassermann lipoid was positive in 923 instances, the Wassermann cholesterol in 1,080 instances, the Kahn in 1,264 instances, and the Hinton in 1,624 instances.

Of the 1,704 serologically positive cases, 54 remained unconfirmed (being positive to one type of serological test only). In 9,000 consecutive cases, 18+ per cent gave positive evidence of having demonstrable syphilis.

CONCLUSIONS

1. On further study the sensitivity of the Hinton test in the serological examination for syphilis is still greater than any of the other tests.

2. Reliance cannot be placed on one serological test alone.

3. Thus far all of our studies substantiate our original premise that all available tests should be utilized in the establishment of the diagnosis of syphilis in order to give the best possible serological service in this disease.

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THE USE OF PARAFFIN RINGS FOR RAPID BLOOD TYPING*

CLARA M. BECTON, M.S., M.T., TULSA, OKLA.

LANDSTEINER¹ and Coca² and others have emphasized the importance of determining the agglutinins of human bloods as well as the agglutinogens when typing blood for transfusion. In order to determine the agglutino-gen-agglutinin distribution in bloods, it is necessary to test the unknown cells with Groups A, B, and O serums and the unknown serum with A and B cells.

A simple method for determining the presence or absence of agglutinogens and agglutinins in blood is the use of paraffin rings in the same manner as for the Kline Precipitation Test on a 2 by 3 inch glass slide. Fifteen paraffin rings, $\frac{3}{8}$ inch inside diameter, are placed on a 2 by 3 inch glass slide, 5 rows long by 3 rows wide, three unknown bloods can be tested at a time with this setup.

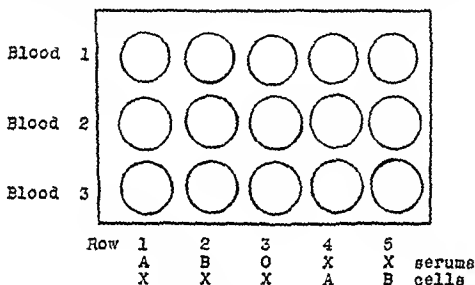


Chart 1—Diagram for paraffin rings

Light cell suspensions are set up in the following manner:

Row 1	A serum	plus	X cells
Row 2	B serum	plus	X cells
Row 3	O serum	plus	X cells
Row 4	A serum	plus	A cells
Row 5	B serum	plus	B cells

The slide is rotated for three minutes and examined with the microscope for agglutination.

The four Landstemei groups give the following reactions with this method

GROUP	A SERUM	B SERUM	O SERUM	A CELLS X SERUM	B CELLS X SERUM
O*	-	-	-	+	+
A	-	+	+	-	+
B	+	-	+	+	-
AB	+	+	+	-	-

*—signifies lack of agglutination

+ signifies agglutination

* Aided by the Mark Finston Research Fund

From the Department of Pathology, St. Johns Hospital, Tulsa

Received for publication July 17, 1937

The examination of blood for agglutinins is a very valuable check on typing. In case the A and/or B test serums are faulty, the use of Group O serum and A and B cells will indicate typing irregularities in most instances. Moreover, this method shows up defective and/or atypical blood groups in that there is a double check for the agglutinogens and agglutinins of the unknown blood.

The A and B cells must be fresh or preserved in a medium which prevents diffusion of agglutinin. Unwashed cells suspended in their own serum and kept at icebox temperatures retain high agglutinin content for a week at a time. The typing serums are standardized according to Weiner.³

This method has proved very rapid in our hands, thus saving much time when large numbers of bloods are to be typed. It is not recommended for compatibility tests. Evaporation must be avoided in the cross-matching of bloods, and only closed chamber or test tube methods are recommended for the actual compatibility test.

SUMMARY

1. A rapid method for blood typing, determining both the agglutinogens and agglutinins, has been described.

2. Paraffin rings are quickly prepared on and removed from glass slides, whereas cover slips require tedious handling if they are employed with slides in a procedure.

3. This method has the advantage over porcelain plates in that each agglutination setup can be examined with a microscope, thus eliminating the possibility of overlooking latent or weak agglutination.

I am indebted to Dr. I. A. Nelson for many valuable suggestions.

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BLOOD GROUPING FOR MAJOR BLOOD GROUPS WITH PLASMA AND OXALATED BLOOD CELLS*

CLARA M. BECTON, M.S., M.T., TULSA, OKLA.

IN a discussion on the various uses of oxalated blood, Dr. I. A. Nelson, Director of Laboratories at St. John's Hospital, called my attention to the fact that there is no basic reason why potassium oxalate would alter the agglutinogens and agglutinins of human blood and that it should be possible to type as correctly with plasma and oxalated cells as with cells and serum.

In this series of experiments, cross matching was not carried out, the object being to compare blood grouping with cells and serum and oxalated plasma and cells. The typing sera and plasma were obtained from the same individuals, and standardized according to Wiener.¹

Five hundred bloods were typed in duplicate with serum and plasma. Precipitation of fibrin in the oxalated plasmas was avoided by eliminating the suspension of cells in saline and/or citrate, the oxalated cells being suspended in their respective plasma. Identical reactions were obtained in this series.

	A SERUM	A PLASMA	B SERUM	B PLASMA	FINAL GROUPING
12 Bloods	+	+	+	+	AB
42 Bloods	+	+	0	0	B
182 Bloods	0	0	+	+	A
264 Bloods	0	0	0	0	O

SUMMARY

Failure to agglutinate, or false agglutination did not occur in 500 bloods typed with plasma and oxalated erythrocytes.

CONCLUSIONS

1. Oxalated blood cells typed with plasma gave the correct blood types.
2. Typing with plasma and oxalated cells saves laboratory time, especially if venous blood is used for routine blood counts, etc.

REFERENCE

1. Wiener, A. S. Blood Groups and Blood Transfusions, p. 13, Baltimore, 1935, C. Thomas.

*From the Department of Pathology, St. John's Hospital, Tulsa.
Received for publication July 17, 1937.
Aided by the Mark Finston Research Fund.

METHOD FOR CLEARING COAGULATED SERUM-BLOCKED BERKEFELD FILTERS

MAURICE VAISBERG, M.D., PATCHOGUE, N. Y.

DURING the course of some serologic procedure, some human serum was filtered through a small Berkefeld. Inadvertently the candle was placed at once into some boiling water for sterilization without taking the precaution of "washing" the serum out of the Berkefeld with water several times.

On attempting to use the candle on a later occasion, it was found that no pressure or suction effect could be obtained. The albumen had precipitated into the interstices of the filter and had blocked it almost completely.

A solution of egg albumen was tried (since precipitated proteins are supposed to redissolve in an excess), but the result was poor. Before trying any other solution through the Berkefeld, a solution of egg albumen was boiled and flocculated out in a test tube and various reagents added to see the effect. The following all increased the flocculation: xylol, acetone, ether, hydrogen peroxide, and anionia. At last Clorox (a 5 per cent solution of sodium hypochlorite) was used. This cleared the egg albumen precipitate immediately.

By using strong continuous suction and full strength Clorox in the filter, the Berkefeld began to function fairly well in about five minutes, and in about fifteen minutes was completely cleared. It was washed through with plain water several times, boiled, and retested. It was then as good as new.

This is a method for clearing Berkefeld filters which have accidentally been blocked by coagulated protein material.

To test the further efficacy and usefulness of the filter the following test was performed: An actively growing seven-day broth culture of a hemolytic streptococcus was used. A minute quantity was transferred to a fresh sterile broth culture tube. The rest (about 7 c.c.), consisting of a very turbid fluid, was filtered through the Berkefeld. The filtrate was absolutely clear. A large amount of the filtrate was transferred to sterile broth for culture. All the cultures were incubated for several days. There was an active growth in the reseeded tube, but no growth in the culture of the filtrate. Hence, by the physical clearing of the filtered material and the sterility of the filtrate, the continued efficacy of the cleansed Berkefeld was demonstrated.

THE DETERMINATION OF ALCOHOL IN BLOOD AND OTHER BODY FLUIDS*

J. W. CAVETT, PH.D., MINNEAPOLIS, MINN.

NUMEROUS methods have appeared during the past few years for the determination of alcohol in blood and the various body fluids. Most of the European methods have been modifications of the Widmark method¹ in which the distillation of alcohol into the standard dichromate sulfuric acid mixture is carried out in a 50 cc glass stoppered Erlenmeyer flask. The bottom of the glass stopper carries a 0.5 cc cup attached by means of a glass rod. One tenth cubic centimeter of the sample to be analyzed is placed in this cup. On standing two to twelve hours, depending on the temperature, all volatile material such as water, alcohol, etc., passes over into the dichromate sulfuric acid mixture. To determine the amount of dichromate which did not react with alcohol, potassium iodide is added and the freed iodine titrated with thiosulphate. The dichromate destroyed by the alcohol indicates the amount of alcohol present in the sample.

It appears that Widmark's type of distillation has been used but little in America. The common practices used to isolate the alcohol from the blood or fluid are: some form of aeration, to add a protein precipitant and distill, or to prepare some type of filtrate and distill. It appears that although a waiting period is not required by the above procedures there is a greater chance for error and more work is involved.

Harger² presented an alcohol method in which he titrated the excess dichromate with ferrous sulphate and methyl orange in a 62 per cent by weight sulfuric acid solution. Methyl orange is destroyed by dichromate but as soon as an excess is added the solution turns pink, indicating the end point. It appeared that this method of titration would have some advantages if used with the Widmark distillation.

APPARATUS

A special 50 cc glass stoppered Erlenmeyer flask. The glass stopper carries a small cup (Fig. 1).

A 0.1 or 0.2 cc pipette graduated between two marks which, to give the desired accuracy, should be about six inches apart.

A 5 cc volumetric pipette.

A 5 cc Mohr pipette attached by a rubber tube to a stopcock and held by a buret clamp serves as an excellent buret for the titration. The tip may be drawn out until drops of 0.02 cc are delivered. The buret is filled by applying suction through a rubber tube attached to the stopcock.

*From the Laboratory of Physiological Chemistry, University of Minnesota.
Received for publication July 27, 1937.

various movements, after seeing a light flash, were timed with a Cenco impulse counter measuring to $\frac{1}{120}$ of a second. Three individuals on which several tests were made with the above apparatus when varying concentrations of alcohol were present in the blood gave the following average results.

0.05 per cent alcohol in the blood slowed the reaction time 7 per cent.
0.08 per cent alcohol in the blood slowed the reaction time 12 per cent.
0.10 per cent alcohol in the blood slowed the reaction time 19 per cent.

Miles⁴ found that lid reflex, eye reaction time and eye movement velocity are slowed up by alcohol. The term "channel driving" has been used to express one's inability to note things approaching from the side when driving under the influence of liquor. In these experiments this phenomenon was noted in that the subjects were less disturbed by outside influences and were more attentive to the light signals after receiving alcohol than during the control determination.

With 0.1 per cent alcohol in the blood the subjects were able to walk a straight line and talk intelligently although they were somewhat talkative.

Brandt⁵ states that most motorcycle accidents occur when the blood alcohol is 0.1 to 0.15 per cent and most automobile accidents when it is 0.1 to 0.2 per cent. Apparently the majority of accidents occur when the reaction time for manipulating the controls of an automobile has been decreased more than 20 per cent.

SUMMARY

A simple method requiring but little special apparatus has been presented for the determination of alcohol in blood, urine and saliva.

A short discussion of its use and interpretation of the results has been given.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TUBERCLE BACILLI, in the Gastric Contents, Stiehm, R. H. Am J M Sc 194 940, 1937

The increasing use of the tuberculin test combined with roentgen ray examination of the infected lung may manifest many cases of asymptomatic pulmonary tuberculosis.

Laboratory procedures frequently make possible determinations of the status of the lesion before the roentgenogram shows a change.

Tubercle bacilli were found in the gastric contents in 15 of 21 (71.4 per cent) minimal cases of pulmonary tuberculosis in which the sputum was negative.

The absence of tubercle bacilli in the gastric contents suggests an inactive lesion, or if active, that the prognosis is good. Retrogression occurred in 5 of the 6 cases in this series without the institution of treatment other than increased hours of rest.

The examination of the gastric contents for tubercle bacilli frequently gives positive evidence (71 per cent in this series) that the minimal infiltration is pathologically active and in need of treatment. The procedure is of inestimable value in helping to determine, especially in the asymptomatic cases, how long active treatment should be continued. One of the criteria of a healed lesion is the repeated absence of tubercle bacilli in the gastric contents as indicated by guinea pig inoculation. Strict adherence to this principle would result in fewer recurrences in the many patients with apparent but not real cures.

ANEMIA, Acute Hemolytic (Lederer Type), Giordano A. S., and Blum, L. L. Am J M Sc 194 311, 1937

Three cases of acute hemolytic anemia (Lederer type) are reported and the literature reviewed.

The general picture of the disease is drawn and the differential diagnosis discussed.

Acute hemolytic anemia is characterized by an acute onset with fever, rapid development of a severe anemia with the appearance of immature cells, marked reticulocytosis and leucocytosis, and tendency to complete recovery. The dramatic response to a single blood transfusion appears to be very characteristic. The anemia is usually of the normochromic type, with marked tendency to macrocytosis.

It is suggested that acute hemolytic anemia is not a rare occurrence, and that many cases of obscure anemia reported in the literature as well as reports on recovery from acute leucemia actually belong to this group.

The clinical recognition of Lederer's anemia is of greatest practical importance since the untreated disease has a high mortality rate while the prognosis of the treated disease is absolutely favorable. Blood transfusion is the treatment of choice.

The reported occurrence of spontaneous autohemagglutination in course of acute hemolytic anemia is observed for the first time.

Some theories as to the action of blood transfusion are briefly presented.

The etiology of acute hemolytic anemia is at present unknown, but its infectious nature is very strongly suggested. A comparison with the acute hemolytic anemia of Oroya fever is made.

ANEMIA, Sickle-Cell, in the White Race, Haden, R. L., and Evans, F. D. Arch. Int. Med. 60: 133, 1937.

Two white patients with sickle-cell anemia are reported.

The patients are sisters, born of Sicilian parents.

There is no history or physical finding suggesting an admixture of Negro blood.

Both patients have had the spleen removed, with marked benefit.

In one case the spleen was removed fourteen years ago and in the other five years ago.

Both patients continue to have mild hemolytic anemia but are clinically well.

In sickle-cell anemia there seem to be two factors in the excessive hemolysis characteristic of the disease, viz., excessive filtration of abnormally shaped cells and excessive fragmentation or early death of the cells.

The results obtained in these cases suggest that splenectomy should be employed more often in sickle-cell anemia if the spleen is large and the patient is seen early in the disease. It is not a cure but only an aid in treatment, since anemia persists.

LEUKEMIA, Monocytic, Montgomery, H., and Watkins, C. H. Arch. Int. Med. 60: 52, 1937.

Distinction is made between monocytic leucemia of the Naegeli and that of the Schilling type on the basis of the hemocytologic pictures. Either type may be of primary autochthonous cutaneous origin. The type of cutaneous manifestations of either condition may be specific or nonspecific and may vary from discrete necrotic nodules or purpuric lesions to generalize exfoliative dermatitis. A distinctive histopathologic picture of monocytic leucemia of the Schilling type may be observed on examination of the skin, corresponding to the hemocytologic picture. Occasionally, myelogenous leucemia may terminate as monocytic leucemia of the Naegeli type, or, as in Case 4, lymphatic leucemia may result in monocytic leucemia (Schilling) and still later again present the blood pictures of both conditions. Monocytic leucemia of the Schilling type (leucemic reticulo-endotheliosis) may assume an acute or chronic form, and even aleucemic reticulosis may be encountered.

It is important, therefore, to correlate the cutaneous as well as the general clinical, pathologic, and hemocytologic observations in a given case in order to arrive at a correct diagnosis. A prolonged period of observation may be necessary before it is possible to ascertain the type of lymphoblastoma that will eventuate.

DIARRHEA, Epidemic, in the New-Born, Rice, J. L., Best, W. H., Frant, S., and Abramson, H. J. A. M. A. 109: 475, 1937.

This report is a preliminary analysis of outbreaks of highly fatal diarrhea that have occurred among newborn babies in the nurseries of eleven lying-in institutions of New York City from July, 1934, to December, 1936. Among 3,672 liveborn babies, 505 cases of the disease occurred, a morbidity rate of 14 per cent. Of the sick infants 234 died, a mortality rate of 6.4 per cent. The case fatality rate was 46 per cent. Extensive bacteriologic investigations failed to reveal the inciting agent in the disorder. Autopsy did not shed any definite light on the pathogenesis of the disease.

The clinical syndrome was characterized by signs of acute intestinal toxicosis, probably of primary origin, and was accompanied by the symptom train of frequent watery yellow stools, abdominal distention, severe dehydration, drowsiness and acidosis, rapid weight loss, little temperature reaction, marked toxicity and prostration. Vomiting occurred occasionally; blood, pus, or mucus in the stools was infrequent. The main complications were terminal otitis media and bronchopneumonia.

From observations of the various outbreaks, and from a careful search of the literature, it is the authors' present impression that they are dealing with a well-defined clinical entity affecting infants of the newborn period of life, deserving consideration apart from other diarrheal disturbances of infancy. They feel that the condition is more widespread than a survey of the literature would lead one to believe.

TETANUS BACILLI, Isolation of, from Street Dust, Gilles, E C J A M A 109 484, 1937

This investigation was undertaken to determine the presence of tetanus spores in street dust. The method employed was to cultivate the material, isolate tetanus like organisms, study their biochemical reactions and, finally, demonstrate the ability of the strains isolated to yield a spasm producing toxin which could be neutralized by tetanus antitoxin.

As a preliminary to the isolation of pure cultures, it was necessary to grow the mixed material in special mediums to stimulate the growth of any tetanus organisms present and then to eliminate the vegetative forms of bacteria by heating the material at 80° C for twenty minutes. Since, however, the spores of sporulating aerobes and facultative anaerobes were present, the use of selective dyes in the medium was found necessary to eliminate them. The dye employed was crystal violet, which was found to be entirely satisfactory. In the separation of the tetanus organism from other spore bearing anaerobes, the method originally employed by Fildes was used. This procedure makes possible the isolation of tetanus organisms from a mixture of anaerobes.

The biochemical reactions of the strains of *Clostridium tetani* isolated were studied, and their toxin producing ability was demonstrated by the inoculation of animals. From the sixty three samples of street dust obtained from various streets within a limited area of Baltimore and examined in this investigation, eleven strains of tetanus bacilli were isolated, that is, 17.4 per cent of the samples revealed the presence of the organism. Of these, nine strains, or 14.2 per cent of the samples, yielded a spasm producing toxin neutralized by tetanus antitoxin, whereas two strains isolated from 3.17 per cent of the samples did not yield any detectable toxin.

The results obtained in this investigation do not permit of comparison with any other work of a similar nature, since apart from the early observations of Nicolaier and Bosmano, no reports have been found in the literature of the isolation of tetanus organisms from street dust, and the present investigation appears to be the first successful isolation of tetanus bacilli from the street dust of a large modern city of the United States.

The positive results are definite and conclusive proof that *Clostridium tetani* is widely distributed in street dust even at the present day and establish the absolute necessity for the use of prophylactic injection of tetanus antitoxin in all cases of street accidents, accompanied by laceration or abrasion of the cutaneous surface. The fear of anaphylaxis can no longer justify the failure to employ protective serum in every case of street accident in which there are open wounds, since street dust, contrary to the views of many, has been shown to be a potential source of danger.

FUNGI, A Specific Chemical Medium for Pathogenic, Southworth, W H Arch Dermat & Syph 36 302, 1937

The preparation of this medium, which may prove of interest to mycologists, is as follows:

Water	1,100 c c
Dextrose	40 g m
Magnesium sulfate	0.5 g m
Potassium dihydrogen phosphate	3.0 g m
Dipotassium hydrogen phosphate	0.5 g m
Sodium chloride	1.0 g m
Agar	15.0 g m
Urea	10.0 g m

Dissolve the constituents, except the urea, in 1,000 c c of water. Put 250 c c of solution into each of four small mouthed bottles. Sterilize the bottles in the autoclave at 15 pounds (68 kg) pressure for twenty minutes. Dissolve the urea in 100 c c of water, and

filter the solution through a Berkefeld filter. Add 25 c.c. of sterile solution of urea to each of the four bottles after they have been sterilized and cooled to about 55° C. Place in test tubes quantities of from 10 to 12 c.c. of the medium before the agar begins to solidify.

A mixture prepared according to these directions has a pH between 5.6 and 5.8

MARROW PUNCTURE, Sternal, in Infants and in Children, Kato, K. Am. J. Dis. Child. 54: 209, 1937.

A differential cell count of nucleated elements seen in bone marrow smears made with aspirated material from infants and children reveals certain characteristics which differentiate their picture from that of the adult marrow. These differences are (1) the relatively high percentage of erythrocytic and lymphoid elements in the marrow of young subjects, and (2) the relatively low percentage of myeloid elements due to a lack of more mature granulocytes. This reversal of the percentage in erythrocytic and myelogenic elements explains the low myeloid-erythroid ratio of 1:2 for infants and young children, older subjects normally having a value of 3:1.

The fact that the diagnosis was made possible by the sternal puncture method in a case of acute myelogenous leukemia and in a case of Niemann-Pick's lipoidosis illustrates the specific value of the method as a diagnostic procedure.

TUBERCULIN, Skin Sensitivity in Chronic Tuberculosis in the Course of Hospital Treatment, Hayes, M. G., Pastor, J. R., Gaetan, L. R., Cory, R. A. S., and Long, E. R. Am. J. M. Sc. 194: 220, 1937.

Fluctuations in the intensity of the tuberculin skin reaction were studied by monthly tests in 116 ward patients with chronic pulmonary tuberculosis. Twenty patients remained under observation approximately one year and 67 for more than four months. To ensure uniformity, standardized tuberculin (Purified Protein Derivative) was used throughout the experiment. Two doses, viz., 0.00002 mg. (the standard first dose) and 0.000002 mg. (one-tenth of the standard first dose) were employed on each patient. In most cases one or the other of these detected the threshold of reaction.

The general level of sensitiveness to tuberculin was low. Strong reactions were never seen. Of the 20 patients remaining under observation approximately a year, not one averaged more than a 2-plus reaction to the stronger of the 2 doses. Eight of the 116 patients were negative to both doses for the whole course of their study. To the stronger dose 27 per cent of the entire group were negative, and 72 per cent to the weaker dose, two-thirds of the time.

Of the 67 patients injected at 5 or more monthly intervals, more than half maintained approximately the same level of sensitiveness, as did 12 out of 20 followed for approximately a year. The fluctuations in the remainder were of inconstant character, but certain consistent trends were observed. While, as is already well known, patients with unfavorable course tended to lose their tuberculin sensitiveness as the end approached, a definite continuing depression of sensitivity was observed also in patients with chronic tuberculosis of long duration, but somewhat favorable course. On the other hand, transient or prolonged increase in sensitiveness occurred in numerous patients with clinical improvement.

Observation was directed particularly toward the effect of special therapeutic procedures employed, and also toward such events as pulmonary spread of the disease, and particularly pleural effusion. Pneumothorax per se had no noticeable effect. In the small number of cases of thoracoplasty observed, a drop in sensitivity followed the operation. In the majority of cases pleural effusion appeared to depress skin reactivity.

No correlation could be established between tuberculin skin sensitivity and the patient's temperature or with seasonal influences.

TUBERCULOSIS, Agglutinins for Typhoid Group Bacilli in, Damon, S. R. Am. J. Hyg. 26: 40, 1937.

Serums from 143 cases of tuberculosis having negative histories of previous enteric infection or T.A.B. inoculation have been analyzed for agglutinins for the H and O antigens of *B. typhosus*, *B. paratyphosus B*, and *B. paratyphosus A*.

Eighty seven (60.8 per cent) of these serums had no demonstrable content of agglutinins for any of these antigens in dilutions of 1:20 or over

Fifty six (38.4 per cent) of the 6 serums contained agglutinins for one or more of these antigens in dilutions of 1:20 or over

The titers for the H antigens frequently exceeded that generally taken as diagnostic (1:100) in persons having no history of infection or vaccination

The titers for the O antigens never exceeded that taken as diagnostic (1:100) in individuals never having had enteric infection or prophylactic vaccination and seldom exceeded 1:20

These results suggest that in the serologic diagnosis of enteric infections in persons suffering from tuberculosis, unreliable results may be obtained in a large percentage of cases when the examination is made by the classical *Widal* test. They also indicate that these misleading results may be obviated by the use of a qualitative receptor analysis

However, following this procedure it is to be noted that the H antigen cannot be employed with serums from tuberculous individuals even though the serums come from patients having negative histories of previous enteric infection or prophylactic vaccination. Ordinarily in serum from such persons, agglutination of the H antigens in diagnostic titer is taken as indicating not only enteric infection but also the species of infecting microorganisms. In serum from tuberculous patients the frequent appearance of non-specific reactions invalidates any such conclusion and we are forced to fall back on the reactions with the O antigens. Therefore we may perhaps conclude that as agglutinins for the O antigens in sufficiently high titer to be significant have never been encountered in our experience in serums from tuberculous patients we may use this reaction to detect definite enteric infection without indication of the infecting species of organism. However, here, as in all such agglutination tests, the demonstration of a rising titer of O antibodies in the patient's serum, on repeated tests carries relatively more weight than the absolute titer in any single examination

TUBERCLE BACILLI in Gastric Washings of Infants and Children *Am J Dis Child* 51:48, 1937

The finding of tubercle bacilli in the gastric contents, as determined by the production of tuberculosis in a guinea pig inoculated with the sediment obtained from the gastric washings, is indicative of the presence of an active tuberculous focus. This lesion is usually either in the lung or in a hilar or tracheobronchial lymph node which has ulcerated into the bronchus

Tubercle bacilli were demonstrated in the gastric washings in 7 (8.14 per cent) of a series of 86 infants and children with positive cutaneous reactions to tuberculin in whom no recognizable evidence of tuberculosis could be demonstrated on physical or roentgenographic examination

Examination of the gastric washings for tubercle bacilli by the inoculation of a guinea pig with the sediment should be done for all infants and children with positive cutaneous reactions to tuberculin, even if no evidence of tuberculosis has been found on physical and roentgenographic examination. The presence of tubercle bacilli in 8.14 per cent of the children in our series in the absence of physical and roentgenographic findings indicates the necessity of such a procedure, since the diagnosis of active tuberculosis could not have been made in these cases without the positive evidence obtained by examination of the gastric contents. These patients would have remained an unrecognized source of tuberculous infection to uninfected children with negative cutaneous reactions to tuberculin

REVIEWS

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Practical Talks on Kidney Disease*

DISEASE of the kidney is a diagnostic and therapeutic problem with which the physician is frequently confronted. It is unfortunate, therefore, that this subject is—and with some justice—regarded as both difficult and confused.

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The book is not intended as a monograph or textbook but rather as a practical presentation of the subject. In this it is exceptionally successful.

It is divided into six main sections in which are discussed: The Mechanism of Kidney Function; Disturbances of Renal Function; Signs and Symptoms of Kidney Disease; Glomerulonephritis; Nephrosis; Pregnancy and Kidney Disease; and Nephrosclerosis.

While the book will prove of value to the student and physician alike, in its preparation Dr. Weiss has performed a service of inestimable value to the practitioner to whom this little volume should prove invaluable.

Genital Abnormalities, Hermaphroditism, and Related Adrenal Diseases†

STATED to be the most comprehensive treatise yet published upon these subjects, this is in many respects a remarkable book.

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The numerous original drawings by William P. Didusch and the many photographs, together with the text, make this an epochal volume which for some time to come will be the outstanding reference in this field.

*Practical Talks on Kidney Disease. By Edward Weiss, M.D., Professor of Clinical Medicine, Temple University School of Medicine. Cloth, 176 pages, 10 figures, \$3.00. Charles C. Thomas, Springfield, Ill.

†Genital Abnormalities, Hermaphroditism, and Related Adrenal Diseases. By Hugh Hampton Young, The Brady Urological Institute, Baltimore. Cloth, 680 pages, 534 illustrations, \$10.00, Williams and Wilkins Company, Baltimore, Md.

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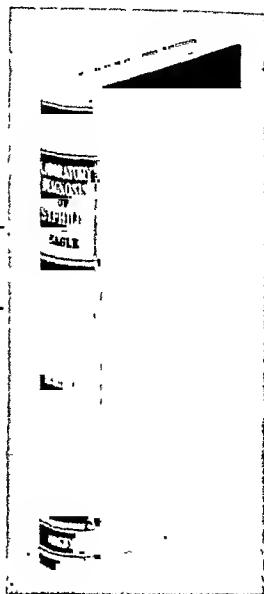
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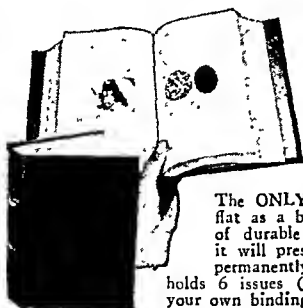
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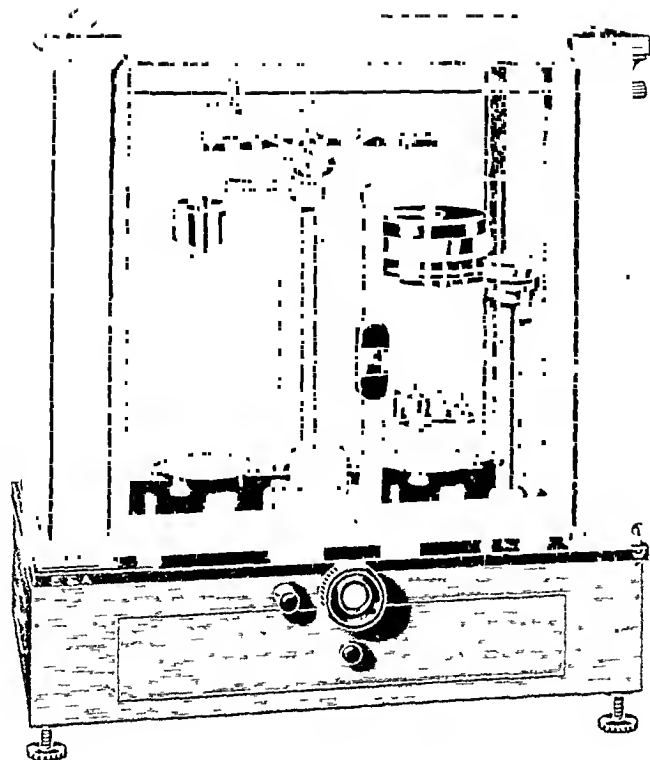
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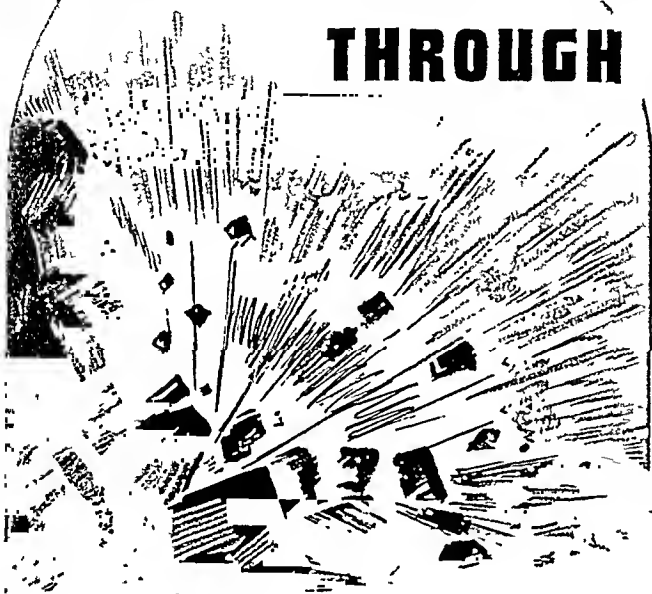
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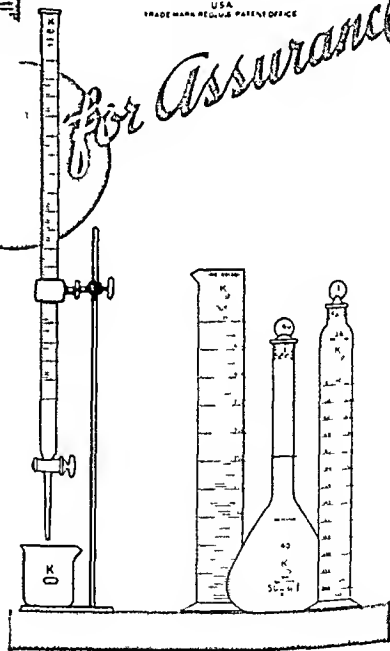
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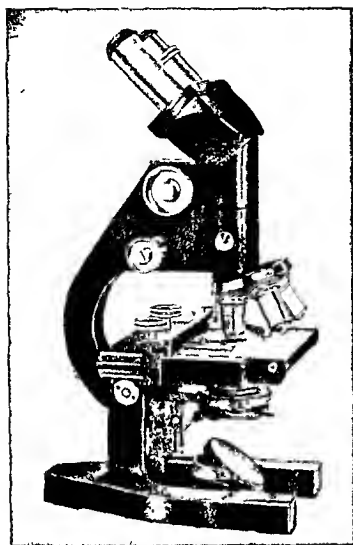
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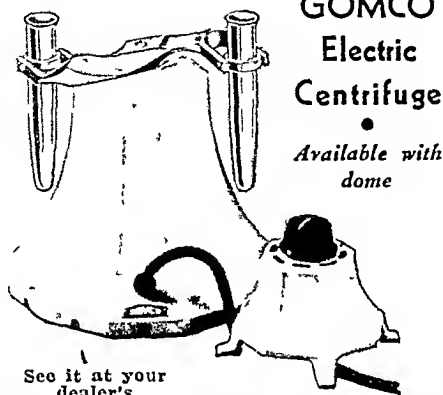
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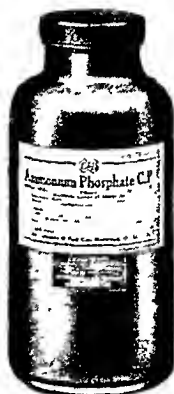
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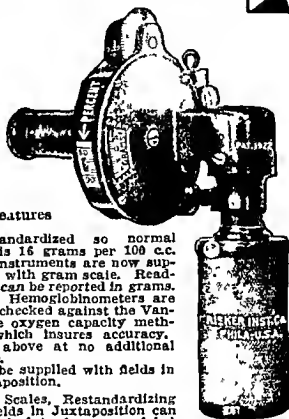
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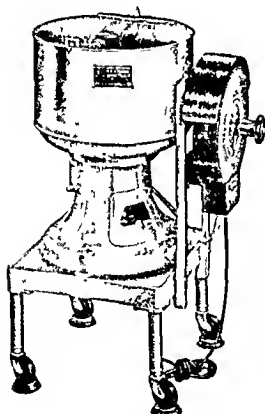
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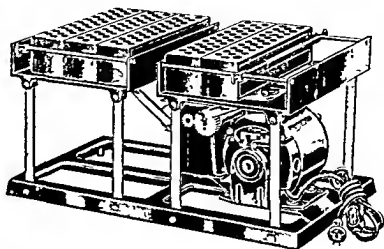
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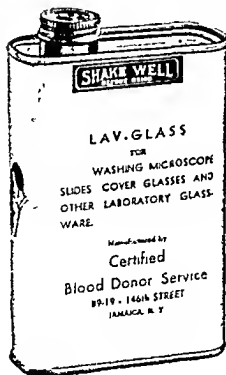
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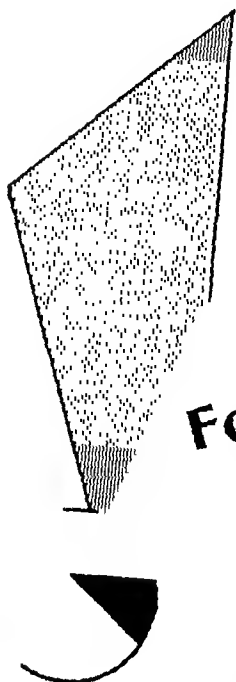
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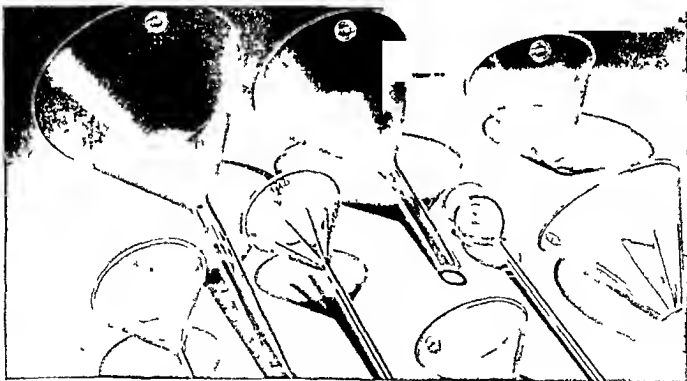
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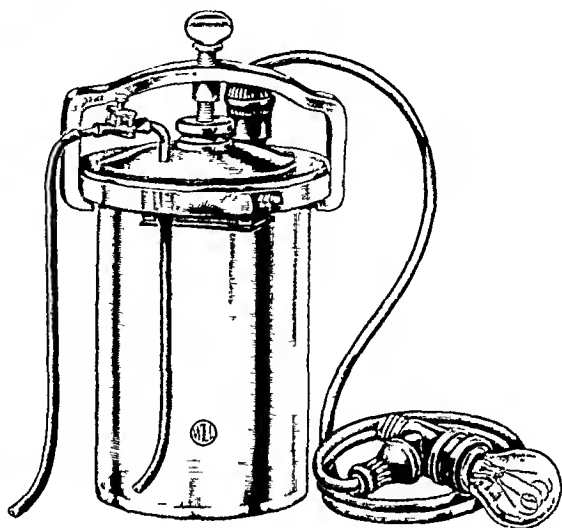
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8 had severe colds, 2 had carcinomatous involvement of the lungs, 3 had chronic nonspecific pulmonary infections, 1 had an undiagnosed pulmonary infection with probable malignancy, 1 patient had esophageal diverticulum with a possible mediastinal abscess, and 2 patients were undiagnosed until mycotic infections were discovered. The patients from the Southern Pacific Hospital all had pulmonary tuberculosis. In addition to the tuberculous infection one patient had pneumoconiosis, one had diabetes, and a third had atrophic arthritis.

Individual specimens were examined for the presence of fungi from one to ten times. The total number of sputum examinations were 373. The specimens varied from single expectorations to specimens collected over a period

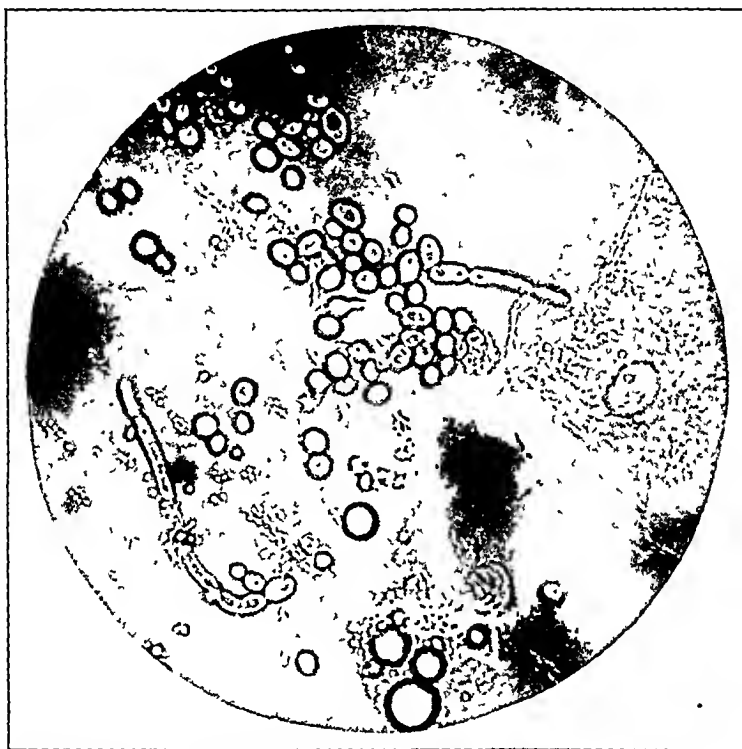


Fig. 1.—Budding form of monilia in fresh sputum, together with fat globules, epithelial cells, and bacteria (Case DrG).

of twenty-four hours. One specimen was a thirty-six-hour collection, the total amount of sputum at that time being 6 gm. The specimens were all collected in closed containers. Each specimen was searched for suspicious particles—the typical small grayish masses analogous to the sulfur granules of actinomycotic infections. These masses contain the mycelium and encysted forms together with fibrin, epithelial cells, and leucocytes. Fig. 1 shows *Monilia albicans* as it appeared in a fresh specimen of sputum (Case DrG). The yeastlike forms and beginning hyphae are seen in the photograph together with epithelial cells, bacteria, fat droplets, and leucocytes. Particles were examined microscopically and cultured directly upon Sabouraud's glucose agar. The presence of these particles, as Stovall and Bubolz¹ have pointed out, tends to associate

them with the exudate and secretions coughed up from the bronchi. Accidental contamination may in this way be ruled out. Accidental contamination may be picked up on culture but rarely on wet preparation.

It was not possible to make a study which included clinical follow-up investigations owing to the nature of the patients seen at the Desert Sanatorium, where admissions are largely seasonal, with many patients coming for the winter months only and remaining in Tucson for a period of from a few weeks to a few months. Some came for diagnostic studies only and were in the clinic for as brief a period as a few days. The available material was utilized, and

TABLE I
CASES WITH DIAGNOSES AND MYCOLOGICAL FINDINGS*

CASE	CLINICAL DIAGNOSIS	FUNGUS ISOLATED FROM SPUTUM	NO. OF SPUTUM EXAMS.	NO. OF POSITIVE EXAMS.
WmS	Pulmonary tuberculosis	<i>M. albicans</i> <i>Endomyces</i>	2	2
MH	Asthma	<i>M. candida</i>	1	1
ACh	Undiagnosed pul. infection with probable pul. malignancy	<i>M. albicans</i>	6	6
GR	Pulmonary tuberculosis	<i>M. albicans</i>	3	3
EP	Pulmonary tuberculosis	<i>M. albicans</i>	8	8
KSP	Pul. tuberculosis Atrophic arthritis	<i>M. albicans</i>	1	1
FrD	Chronic nonspecific pul. infections	<i>M. albicans</i>	6	6
DrG	? Pul. tuberculosis ? ? Lymphogranuloma ?	<i>M. albicans</i>	5	5
WmSh	Sinusitis	<i>Saccharomyces</i>	1	1
			7	3
			5	5
			1	0
			1	1
		<i>Aspergillus</i>	10	10
		<i>Aspergillus</i>	4	3
			2	1
			6	6

*This chart lists examinations performed before any form of antimycotic treatment was administered.

this study is of necessity a clinical laboratory study, and not a combined clinical and laboratory study. The only case in which an autopsy was performed was in the case of HS.

RESULTS

In this series of 141 patients, 17 had positive mycological findings. *Aspergilli* and *coccidioides* were each found 3 times. *Monilias* were found in the sputum of 8 patients; *sporothricum*, *endomyces*, *torula*, and *saccharomyces* were found in the sputum of 3 patients, 2 patients having double mycological findings. In the sputum of one patient *torula* and *saccharomyces* were found, in the other *monilia* and *endomyces*. Table I shows the cases with their original

diagnoses, the mycological findings together with the number of times each sputum was examined and found positive. Because it is not possible in this series of cases, with but two exceptions, to determine whether or not the fungous organism was the etiologic factor in the pulmonary infection, the mycological diagnosis as the disease entity is not included in the chart. In most cases the fungi were in all probability secondary invaders, but that question is entirely outside the scope of this report.

ANALYSIS OF ORGANISMS STUDIED

Yeastlike Organisms.—The yeastlike organisms, saccharomyces, torula, endomyces, and monilias are an important group. In view of the reports of Maher and Maret^t it was surprising to find monilias in so few of the sputa. Maher² reported monilias in the sputum from all of his cases of pulmonary

TABLE II

CULTURE	TYPE ACCORDING TO FERMENTATION REACTIONS	DOSE IN MILLIONS PER 100 GRAM RABBIT WT.	RESULT	POSTMORTEM FINDINGS
WmS	<i>M. albicans</i>	1.5	Died 48 hr.	Generalized moniliasis
MH	<i>M. candida</i>	0.5	Well 6 wk.	No evidence of moniliasis
		2.5	Well 8 wk.	
		6.0	Well 4 mo.	
		25.0	Died 21 days	Generalized moniliasis
ACh	<i>M. albicans</i>	0.5	Died 6 days	Generalized moniliasis
GR	<i>M. albicans</i>	0.75	Died 6 days	Generalized moniliasis
EP	<i>M. albicans</i>	0.5	Died 6 days	Generalized moniliasis
KSP	<i>M. albicans</i>	0.5	Well 6 wk.	No evidence moniliasis
		1.5	Died 5 days	Generalized moniliasis
FrD	<i>M. albicans</i>	0.5	Well	No evidence moniliasis
		1.5	Killed 6 wk.	Mycelia and cysts in bile Generalized moniliasis
		3.0	Killed 9 wk.	
DrG	<i>M. albicans</i>	0.66	Died 72 hr.	Generalized moniliasis
RC	<i>M. albicans</i>	1.0	Died 96 hr.	Generalized moniliasis

tuberculosis when he used milk as a medium. Maret^t states that he grew monilias in one hundred per cent of his cases of tuberculosis. In another series of cultures Maret^t found fifty per cent of all of his sputum specimens to contain monilias.

Seven of the monilias isolated in this series of cases were *M. albicans*, one was *M. candida*. A classification of the monilias was first made according to the classification of Stovall and Pessin⁵ by pathogenicity tests for the rabbit by intravenous inoculation of a measured number of organisms. They found that *M. albicans* was pathogenic for the rabbit in doses of from 0.5 to 1.5 million organisms per hundred gram of body weight. *M. candida* requires doses of from 6 to 35 million organisms per centigram of weight. *M. parapsilosis* is not pathogenic for the rabbit in any dosage. The results of the pathogenicity tests of monilias isolated in this survey are shown in Table II. One culture (strain MH) required 25 million organisms per hundred gram of weight to kill the rabbit, thus classifying the organism as *M. candida*. All the other strains were *M. albicans*.

Stovall and Pessin⁵ have carefully described the pathologic findings in experimental moniliasis in rabbits by intravenous inoculation. The lesions were distributed throughout the body, involving the skeletal muscle, brain, spleen, lungs, with an intensity of reaction in the kidneys and relatively slight, if any, involvement of the liver, although the bile nearly always shows abundant yeastlike cells and many long mycelia.

The finding of monilias in the bile of the experimental rabbits in this series was fairly constant. Strain FrD which was of low virulence for the rabbit produced no gross lesions when inoculated in relatively small doses. One animal receiving 0.5 million organisms per hundred gram of its weight was sacrificed six weeks later. There were no gross lesions. Monilias were

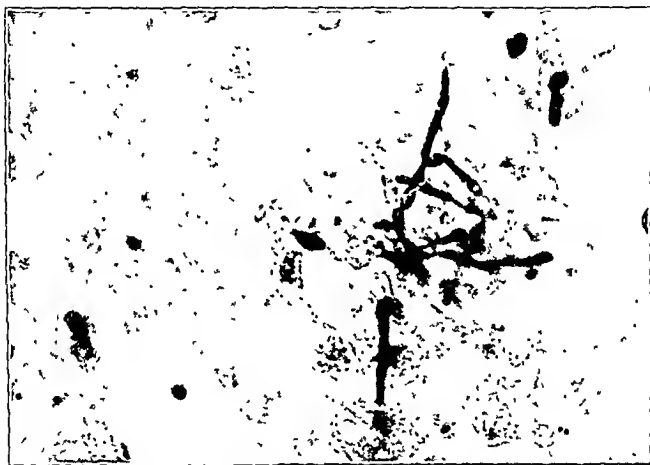


Fig. 2—*M. albicans* in liver of rabbit (Culture WmS).

not recovered on culture from the kidneys, lungs, brain, or bile. A second rabbit inoculated with 1.5 million organisms per hundred gram rabbit weight was sacrificed nine weeks later. The bile was found to contain, on microscopic examination, a few organisms, both cells and mycelia. This was the sole positive finding in this animal. Cultures of the urine, kidneys, lungs, heart, and brain yielded no monilias. It required 3 million organisms of this strain per hundred gram weight to produce generalized moniliasis and ensuing death of the animal. Table II shows the lethal dosage of the monilias isolated in this study. The only organism at variance with the classification of Stovall and Pessin was this organism from the sputum of FrD.

With the strain WmS death was produced in the rabbit in forty-eight hours by intravenous inoculation of 1.5 million organisms per hundred gram of the rabbit's weight. In this case the liver showed small abscesses contain-

ing a large number of organisms. The entire outer surface of the liver had a necrotic appearance. Fig. 2 shows a section of the liver containing the organism. With the strain KSP the liver of the experimental animal showed considerable enlargement. It weighed 116 gm., which was 6 per cent of the animal's total weight. (The weight of a normal rabbit liver as measured in this laboratory is about 2.5 to 3 per cent of the animal's total weight.) Gross



Fig. 3.—Kidneys of rabbit inoculated with *M. albicans* (Culture KSP).

lesions were not numerous in this animal's liver, but by histologic examination many organisms could be found. Fig. 3 shows the kidneys of this same animal. The kidneys containing innumerable small abscesses were enlarged to more than twice the normal size. Fig. 4 in comparison shows the kidneys of an animal inoculated with *M. candida* (strain MH). The pinpoint abscesses are rare. The kidneys are not enlarged.

One of these cases (Case RC) had what appeared to be a primary monilia infection. The patient had the typical severe paroxysmal cough, with large

amounts of tenacious, purulent sputum in which *M. albicans* was repeatedly found. No tubercle bacilli were found on smear by culture or by animal inoculation. He showed toe and finger nail involvement characteristic of this disease. After iodide therapy, his sputum decreased in quantity, changed in character from purulent to mucoid. *M. albicans* could no longer be found on smear nor by culture of concentrated sputum.

The torula organism, another of the yeastlike organisms isolated in this survey, was the red or pink variety characterized by the deep salmon-pink color of the culture. The organism was isolated from the sputum of a young man (Case WmS) with sinusitis. A *saccharomyces cerevisiae* was isolated from the sputum at the same time. The red torula is apathogenic to rabbits by intraperitoneal and intravenous inoculations, and to mice and guinea pigs by intraperitoneal inoculations of large doses. Henri⁶ has called red torula one of the constant contaminants of the bacteriologic laboratory. This, however, was the only time that the red variety of torula was encountered in this laboratory.



Fig. 4.—Kidney of rabbit inoculated with *M. candida* (Culture MH).

Red torula has been reported elsewhere in sputum.⁷ Stovall and Bubolz have also found the "pink" variety in sputum. Taber⁸ reports a fatal case of pulmonary infection with the salmon-pink torula in which the organisms were recovered from the lungs at autopsy.

Saccharomyces also may be found in sputum, but little can be found in the literature of this country regarding its possible etiologic rôle. It is sometimes found associated with other yeastlike organisms. It has frequently been found in cultures of the tongue, tonsils, and teeth in this laboratory. Two series of cultures isolated from sputum were sent to this laboratory from other laboratories in which *saccharomyces* was associated with another yeastlike organism. In each case it was in combination with endomyces.

The endomyces isolated from the sputum of WmS was of low virulence for laboratory animals. It failed to infect mice by intraperitoneal inoculation, guinea pigs by subcutaneous and intraperitoneal inoculation, and rabbits by intravenous inoculation. An intracardiac inoculation into a guinea pig pro-

duced death in two weeks. The lungs showed multiple abscesses from which the organism was recovered. Histologic examination of the lung tissue revealed the yeastlike forms only. The culture was characterized by heavy mycelia, many yeastlike forms, and a few spores. The spores could be demonstrated after thirty-three days' growth on carrot agar.

Sugar fermentation tests were made on all the yeastlike organisms isolated in this study. At the same time, fermentation reactions were determined on stock cultures of *M. albicans*, *M. candida*, *M. parapsilosis*, *torula histolytica*, *endomyces* and *saccharomyces cerevisiae* obtained from Dr. W. D. Stovall, of the University of Wisconsin. At first a wide variation of reactions was produced, not only with the recently isolated strains but with the stock cultures as well. It may have been the age of the culture or the size of the inoculum which was responsible for the variation in reactions as suggested by Hopkins and Hesseltine.⁹ They found that both of those factors, together with the personal equation of the reader, caused a variation in final results.

Stovall and Bubolz¹ reported a constancy of fermentation reactions with 250 cultures of monilias from which they reduced the heretofore complex classification to three species. Fineman¹⁰ also classified the monilias of thrush by fermentation tests and reported constant reactions over a long period. Dodge¹¹ and Dalmau¹² are both of the opinion that sugar reactions are variable, certain strains even losing their power under certain conditions to ferment sugars which once they did ferment. Lamb and Lamb¹³ have employed biochemical and sérologic methods for differentiating and correlating the various species of monilias. They likewise have placed all the monilias into three groups, although their grouping did not coincide with that of Stovall and Bubolz. They agree, however, that *M. parapsilosis* is a group entity. Reed and Johnstone¹⁴ isolated 31 strains of yeastlike organisms from the stools of 50 patients. Twenty of these were monilias. They classify their monilias into Stovall's types I, II and III and add types IV, V, and VI of their own classification on the basis of sugar fermentation reactions. In their study animal inoculations were not made. Boné¹⁵ combined the two classifications by placing a series of cultures isolated from human sources into groups II and III of Stovall and Bubolz by fermentation reactions, and into group I of Lamb and Lamb by serologic tests. Boné did not isolate any organisms of *M. parapsilosis* group in his study of human material, nor was *M. parapsilosis* isolated in this work with sputum.

When the technique of Stovall and Bubolz¹ was followed in this laboratory, using purified sugars in a 1 per cent solution in broth made of Leibig's extract, bromthymol blue as an indicator, and adjusting the pH to 7.2, results were obtained compatible with those of Stovall and Bubolz, who have greatly simplified the classification of the monilias and other yeastlike organisms. Pfau-stiehl's sugars were used throughout the experiment. The growth of a forty-eight-hour culture on Sabouraud's glucose agar slant was suspended in 6 c.c. of sterile distilled water. One-tenth of a cubic centimeter of this suspension was inoculated into each sugar tube and into milk. Milk medium made according to Stovall and Bubolz¹ with the addition of calcium lactate was used for the determination of coagulation. Many series of tests were made employing this

technique Varing lots of Pfanstiehl's sugars were used, such as old stock sugars which had been in this laboratory and in the bacteriologic laboratory of the University of Southern California Medical School for several years, and freshly purchased sugars from several different chemical houses With each "run" the results were identical for all practical purposes Occasionally a strain would show production of a trace of acid in dextrin

The reactions of the yeastlike organisms in dextrose levulose mannose, maltose, galactose sucrose, lactose, dextrin, inulin, raffinose, and milk were determined to aid in the differentiation of the organisms It was decided that the reaction in lactose dextrose, levulose, maltose, sucrose, and milk were sufficient, however for diagnostic procedures, and the use of the larger number of sugars was discarded The monilias, Stovall's types II and III, were easily differentiated by sugar reactions and coincided with the classification already made by the animal pathogenicity experiments as well as with the reaction of the stock cultures of Dr Stovall

The endomyces was differentiated from a monilia by sugar fermentation reactions before the formation of ascospores could be demonstrated *M candida* and *M albicans* both produced acid and gas in maltose while the endomyces showed neither acid nor gas production In this study the red torula was easily classified by its characteristic bright pink color and sticky viscous growth on culture media and morphologic appearance of oval cells, producing neither mycelia nor asci This particular culture did not produce pseudomycelia It fermented dextrose, levulose, mannose, and sucrose without the production of gas Maltose was not fermented The saccharomyces on the other hand, fermented dextrose, levulose mannose, sucrose, and maltose with the production of gas Lactose was not fermented by any of the yeastlike organisms isolated in this group The sugar fermentation tests were of definite value in the classification of these organisms isolated from sputum

Aspergilli.—Of the three aspergilli isolated in this survey two were identified as *Aspergillus fumigatus*, according to Dodge¹² by cultural and morphological characteristics Aspergilli were found consistently over a period of two years in the sputum of two patients In three of the four specimens of WmII sent to the laboratory aspergilli were found Between the second and third specimens, a period of fourteen months had elapsed The sputum still showed aspergilli but was negative for tubercle bacilli by animal inoculation Seventeen months later, when the patient had been pronounced clinically cured of tuberculosis for nearly two years, he sent another specimen of sputum to the laboratory for examination for acid-fast bacilli At that time no aspergilli could be found by wet preparation nor by cultural methods In this case the aspergilli remained in the sputum longer than the tubercle bacilli

The sputum of CK was examined many times over a period of two and a half years and found to contain aspergilli on all occasions The patient had large tuberculous cavities When first examined here, the sputum contained large numbers of tubercle bacilli, caseous particles, elastic fibers and numerous aspergilli Examinations made every month for the next eight months continued to reveal aspergilli in the sputum The patient left the sanatorium

these abscesses yielded *Coccidioides immitis*, pure cultures of which produced typical lesions in guinea pigs, mice, and rabbits when inoculated intraperitoneally.

The second case of coccidiosis (GZ) was the only patient in this series in whom a fungous disease was suspected. The patient came to the sanatorium referred by a physician from an eastern state. The physician had diagnosed his case as pulmonary tuberculosis, complicated by a mycotic infection of unknown origin. The referring physician had noted in the sputum the presence of "granules," which he said contained pus, very few organisms, and no acid-fast bacilli. An "unidentified mold" had been cultured from one of these "granules."

Upon arrival here several weeks later no such "granules" could be demonstrated in the patient's sputum. The sputum was negative for fungi by microscopic and cultural examinations. Acid-fast organisms were present in large numbers. These organisms produced typical inoculation tuberculosis in guinea pigs. The patient seemed better and left the sanatorium, and was placed under the care of the outpatient department.

Five months later a granuloma appeared on his leg. Cultures from this lesion yielded *Coccidioides immitis* in pure culture. His sputum was again examined, this time revealing the typical double contoured cysts of coccidioides.

The third case (PT) of coccidioides was that of a seventeen-year-old boy. He had a slight cough following what he said was a cold, but no other complaints, and said that he felt fine. A roentgenologic examination revealed what appeared to be a rapidly developing pulmonary tuberculous cavity. Repeated examinations revealed no tubercle bacilli. In this case no "granules" were seen. The sputum was concentrated with sodium hydroxide. Coccidioides cysts were found in the concentrate. Fig. 5 shows a group of coccidioides cysts beginning to grow, together with some long mycelial threads, the sputum acting as a culture medium. Cultures on Sabouraud's glucose agar of bits of the sputum picked at random yielded many colonies of *Coccidioides immitis*. Guinea pigs inoculated with the culture as well as with fresh sputum showed typical inoculation coccidioidosis.

Sporothricum.—The sporothricum culture is a little difficult to explain. The organism was isolated from a localized abscess at the site of inoculation of a guinea pig injected with a fresh, undigested specimen of sputum. Fig. 6 shows a culture of the organism with its characteristic stemmed, pear-shaped conidia. The patient (MC), an elderly woman, had complained of respiratory infections over a period of several years. She entered the sanatorium complaining of extreme fatigue, anorexia, and a constant cough which was mostly nonproductive. She remained in the sanatorium for two weeks. Only one specimen of sputum was sent to the laboratory. This specimen was negative for tubercle bacilli both by animal inoculation and by culture. The roentgenologic findings did not rule out a mycotic infection. The patient did not return after this first admittance to the sanatorium. At autopsy of the guinea pig inoculated with the sputum of MC no tuberculous lesions could be found. There was an abscess at the site of inoculation, cultures of which yielded the sporothricum. It is not possible to state definitely whether the sporothricum came from the sputum or was a secondary invader into the

guinea pig tissue *Sporothium* is found with great difficulty in wet preparations and undoubtedly would have been overlooked in the microscopic examination of the wet preparation

SUMMARY

Mycological examination of the sputum of 141 patients is reported. Seventeen patients harbored fungi in their sputum. Specimens were examined from one to ten times making a total of 373 specimens examined. Yeastlike organisms were most commonly found. *Coccidioides* appeared three times. Two patients had double findings, endomyces and *W. albicans*, and saccaromyces and torula. A sporothium was isolated under peculiar circumstances. Two patients showed the presence of *aspergilli* in their sputa over a period of two years. Fermentation tests, morphologic structure, and animal pathogenicity methods were used in classifying the various types of fungi isolated.

I wish to acknowledge my indebtedness to Dr John F Kessel of the Medical School of the University of Southern California who made the completion of this report possible.

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BLOOD COPPER AND IRON IN RELATION TO MENSTRUATION*

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THE effect of menstruation upon the composition of the circulating blood has recently received much attention. Of special interest has been the work of those investigators attempting to correlate hemoglobin and erythrocyte variations with different periods of the menstrual cycle. The findings of different workers are not in agreement. Several investigators including Merletti¹ and Reinl,² have found a decrease in the number of erythrocytes in the premenstrual period; another group which includes Blumenthal,³ Detre,⁴ Holler, Melicher, and Reiter,⁵ Krutschenkoff,⁶ Pölzl,⁷ and Reinert,⁸ have reported a compensatory increase in the number of red cells in the premenstrual phase followed by a decrease when the active flow begins. Four of the most extensive and representative studies, those of Leverton and Roberts,⁹ Smith,¹⁰ Reich and Green,¹¹ and Gumpnich,¹² have shown no constant change which might be correlated with any part of the menstrual cycle.

We have, in the present study, determined erythrocyte counts and in addition the blood iron and blood copper values† for 10 normal women at various stages throughout the menstrual cycle. We feel justified in regarding the results of the blood iron and blood copper determinations as a more accurate index of the changing blood picture than erythrocyte counts and hemoglobin values determined by clinical hemoglobiometers.

METHODS

The subjects used in this work were young women between the ages of twenty and thirty-five. Most of them were laboratory technicians and nurses. All were in good health.

Samples for analysis were taken (1) on different days of the active flow, (2) at about five days after menstruation in the postmenstrual period, and (3) in the intermenstrual period midway between menstruations. The blood was drawn during a preabsorptive period at the same time each day to eliminate possible diurnal variations.

Copper and iron analyses were made on 5 c.c. samples of blood drawn from the cubital vein, and the iron results were checked by another iron method on a 0.5 c.c. sample. Details of the methods for copper¹³ and for iron^{14, 15} determinations have previously been described.

Special precautions were taken to obtain pure reagents, free from copper and iron. Acids, alkalies, and water were redistilled from glass and were tested frequently for contamination.

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†Blood iron and copper in all our work signifies iron and copper values as determined on whole blood unless otherwise stated.

Blood counts were made with standardized pipettes and counting chambers on blood drawn by cutaneous puncture of the lobe of the ear. Hemoglobin values were calculated from the blood iron content, iron constitutes 0.335 per cent of the molecular weight of hemoglobin¹⁰

EXPERIMENTAL DATA

In Table I are recorded for each subject the part of the menstrual cycle during which the sample was taken, the red cell count, the iron content, the

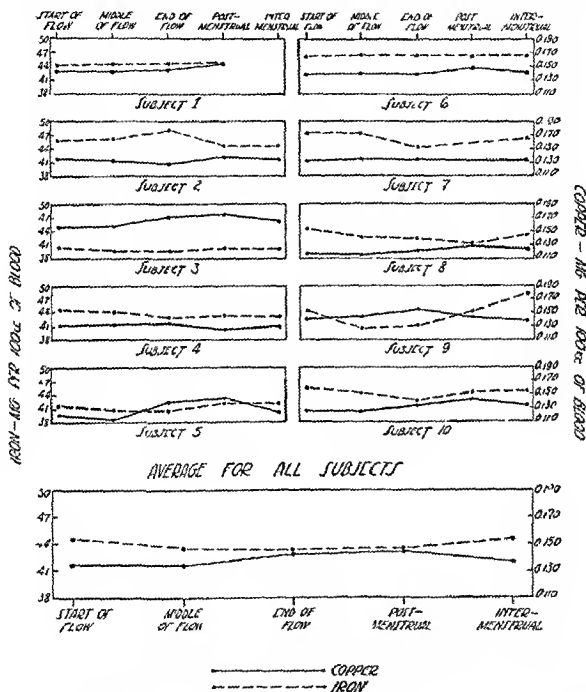


Chart 1—Chart showing whole blood copper and iron values for ten normal women with relation to their menstrual cycles. Curves are charted for each individual and a composite curve representing averages of values for all subjects is included

copper content, and hemoglobin in grams calculated from the blood iron. It is also stated in each case whether the flow was scanty, moderate, or excessive

DISCUSSION

The results indicate that there is a slight decrease in the blood iron during the active menstrual discharge. The decrease is so slight, however, that it can scarcely be regarded as significant, the extremes in the average figures corresponding to little more than 1 mg. of iron per 100 cc of blood

TABLE I

	RED CELLS PER C. MM.	HEMOGLOBIN GM. PER 100 C.C.	IRON MG. PER 100 C.C.	COPPER MG. PER 100 C.C.
<i>Subject 1</i>				
Period—5 days in duration				
Quantity—normal				
First day of flow	4,540,000	13.21	44.25	0.143
Middle of flow	5,120,000	13.29	44.54	0.142
End of flow	4,450,000	13.46	45.11	0.149
Postmenstrual (5 days after flow)	4,480,000	13.43	45.00	0.153
Intermenstrual	-----	----	----	----
<i>Subject 2</i>				
Period—5 days in duration				
Quantity—normal				
First day of flow	4,700,000	13.75	46.08	0.137
Middle of flow	5,130,000	13.91	46.61	0.132
End of flow	4,700,000	14.46	48.45	0.130
Postmenstrual (5 days after flow)	4,670,000	13.32	44.62	0.140
Intermenstrual	4,290,000	13.38	44.82	0.136
<i>Subject 3</i>				
Period—5 days in duration				
Quantity—normal				
First day of flow	4,540,000	12.12	40.63	0.156
Middle of flow	4,350,000	11.89	39.85	0.159
End of flow	4,420,000	11.90	39.88	0.171
Postmenstrual (5 days after flow)	4,330,000	12.09	40.53	0.176
Intermenstrual	3,940,000	12.06	40.40	0.166
<i>Subject 4</i>				
Period—4 days in duration				
Quantity—normal				
First day of flow	4,670,000	13.29	44.54	0.133
Middle of flow	4,420,000	13.14	44.02	0.134
End of flow	4,450,000	12.87	43.12	0.136
Postmenstrual (5 days after flow)	4,480,000	13.01	43.59	0.127
Intermenstrual	4,510,000	12.93	43.33	0.130
<i>Subject 5</i>				
Period—6 days in duration				
Quantity—excessive				
First day of flow	4,670,000	12.51	41.92	0.122
Middle of flow	4,580,000	12.22	40.96	0.114
End of flow	4,510,000	12.18	40.82	0.141
Postmenstrual (5 days after flow)	4,740,000	12.64	42.36	0.146
Intermenstrual	4,510,000	12.65	42.38	0.125
<i>Subject 6</i>				
Period—3 days in duration				
Quantity—scanty				
First day of flow	4,700,000	13.79	46.22	0.141
Middle of flow	4,480,000	13.88	46.50	0.141
End of flow	4,380,000	13.86	46.45	0.141
Postmenstrual (5 days after flow)	4,480,000	13.83	46.35	0.149
Intermenstrual	4,610,000	13.78	46.18	0.143
<i>Subject 7</i>				
Period—6 days in duration				
Quantity—normal				
First day of flow	4,740,000	14.26	47.78	0.133
Middle of flow	4,670,000	14.12	47.30	0.137
End of flow	4,740,000	13.18	44.16	0.137
Postmenstrual (5 days after flow)	-----	----	----	----
Intermenstrual	4,640,000	13.93	46.67	0.134

TABLE I—CONT'D

	RED CELLS PER C MM	HEMOGLOBIN GM PER 100 CC	IRON MG PER 100 CC	COPPER MG PER 100 CC
<i>Subject 8</i>				
Period—4 days in duration				
Quantity—normal				
First day of flow	4,800,000	13.31	44.58	0.118
Middle of flow	4,320,000	12.78	42.82	0.117
End of flow	4,770,000	12.71	42.59	0.124
Postmenstrual (5 days after flow)	4,350,000	12.93	41.93	0.129
Intermenstrual	4,380,000	12.92	41.51	0.124
<i>Subject 9</i>				
Period—3 days in duration				
Quantity—excessive				
First day of flow	3,940,000	13.24	44.38	0.141
Middle of flow	3,680,000	12.15	40.71	0.144
End of flow	3,900,000	12.24	41.06	0.154
Postmenstrual (5 days after flow)	4,640,000	13.19	44.18	0.144
Intermenstrual	4,890,000	14.52	48.66	0.137
<i>Subject 10</i>				
Period—4 days in duration				
Quantity—slightly excessive				
First day of flow	4,450,000	13.56	45.44	0.127
Middle of flow	4,030,000	13.21	44.27	0.125
End of flow	4,420,000	12.78	42.84	0.133
Postmenstrual (5 days after flow)	4,250,000	13.22	44.33	0.147
Intermenstrual	4,480,000	13.39	44.87	0.134
<i>Average for all subjects</i>				
First day of flow	4,580,000	13.30	44.38	0.1351
Middle of flow	4,180,000	13.06	43.76	0.1345
End of flow	4,470,000	12.96	43.45	0.1415
Postmenstrual (5 days after flow)	4,560,000	13.01	43.59	0.1452
Intermenstrual	4,470,000	13.28	44.51	0.1365

The averages of the erythrocyte counts likewise show only small variations, the difference between the highest and lowest of the average counts being 110,000.

Looking at the subjects individually there is also a remarkably small variation in blood iron values. In the majority of the subjects the extremes of variation did not exceed 2 mg and in every case except one, the variation was less than 4 mg of iron and less than 1 gm of hemoglobin per 100 cc of blood. Duckles and Elvehjem¹⁷ have recently reported an average hemoglobin variation of 0.94 grams during the menstrual cycle in a series of seven women. It appears that the individuals showing the most appreciable drops in blood iron during the flow are those who have lost the largest amounts of blood during their menstrual period. The blood loss in menstruation may be considerable, as has recently been shown by Bajer, Fowler, and Baldridge.¹⁸ Their results, based on determinations made during menstruation in 50 normal women, indicate that the blood loss varies from 3.84 to 78.40 mg of iron per menstrual period. Assuming that the iron was derived entirely from the hemoglobin, which assumption is correct for all practical purposes, these figures represent 1.146 to 23.403 grams of hemoglobin lost per period, which is roughly equivalent to 9.39 to 207.28 cc of blood, with an average of 36.70 cc loss per menstrual period.

It seems correct to assume that in most individual cases and in averages of a representative number of cases, the iron, hemoglobin, and erythrocyte values show no decided waves coinciding with phases of the normal menstrual cycle. In a minority of cases where the periodic blood loss is far greater than the average, there may be an appreciable lowering of the blood iron and a mild anemia. The fact that Reich and Green¹¹ found no increase in the number of reticulocytes after the menstrual flow need not be considered contradictory to the finding of an occasional lowered blood iron, because the anemia in such instances is too mild and transitory to demand increased hematopoiesis to offset it.

The blood copper values show comparatively little change during the menstrual cycle. The averages disclose a very small increase in blood copper

TABLE II

CASE NO.	RELATION TO ONSET OF MENSTRUAL FLOW	COPPER, MG. PER 100 C.C.	IRON, MG. PER 100 C.C.	RED CELLS PER C. MM.	HEMOGLOBIN,* GM. PER 100 C.C.
1	20 hours before	0.134	44.87	4,480,000	13.39
	5 days after	0.143	44.33	4,352,000	13.23
2	14 hours before	0.150	44.35	4,760,000	13.24
	14 days after	0.147	45.28	4,830,000	13.51
3	24 hours before	0.127	43.54	4,768,000	13.00
	14 days after	0.133	42.44	4,672,000	12.66
4	2 hours before	0.120	43.77	4,480,000	13.06
	14 days after	0.130	45.67	4,224,000	13.63
5	20 hours before	0.129	46.92	4,576,000	14.01
	14 days after	0.121	47.10	4,832,000	14.06
6	16 hours before	0.149	44.27	4,290,000	13.21
	14 days after	0.137	45.09	4,608,000	13.45
7	8 hours before	0.125	41.88	4,480,000	12.50
	14 days after	0.148	43.41	4,360,000	12.95

*Hemoglobin calculated from the Butterfield factor¹² by the formula mg. of iron per 100 c.c. blood divided by 3.35 equals grams of hemoglobin per 100 c.c. blood.

at the end of the period of flow and in the postmenstrual phase, but the increase is too slight to justify the existence of hypercupremia. At its highest it is well within the physiologically normal average range previously established in our series on normal adults.¹³ In Case 3 the copper values were somewhat increased. This subject, however, had the lowest blood iron in the group, the figure falling below 40 mg. per 100 c.c. of blood, and indicating a mild anemia. We have previously shown¹³ that in anemia the blood iron goes down and the blood copper rises. Hence the increased copper in the above case is very likely due to the anemia and probably has no special relationship to menstruation.

Sarata¹⁹ has reported in 4 cases a very marked increase in copper in blood from women taken within twenty-four hours preceding the onset of menstruation as compared with samples taken midway between menstrual periods. We have accordingly performed another series of analyses on a

small group comparing the copper and iron values of blood samples taken less than twenty four hours preceding the onset of menstruation with samples taken in the intermenstrual period. The results are recorded in Table II.

On the basis of the 7 cases cited here no appreciable change can be noted in either the blood copper or iron in a comparison of values on the same subjects taken in the twenty four hour period preceding menstruation and in the intermenstrual period.

SUMMARY

Blood iron and copper determinations, red cell counts and hemoglobin determinations were made on a series of women in various periods of the menstrual cycle.

No significant changes could be noted which coincided with any definite phase of the menstrual cycle, although a slight decrease in iron and hemoglobin was noted in the period of menstrual flow followed by a slight rise in blood copper in the postmenstrual period. We have found no increase in blood copper within the twenty four hours preceding the onset of menstruation.

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THE INFLUENCE OF THE QUANTITY OF SERUM BILIRUBIN ON THE TYPE OF VAN DEN BERGH REACTION^{*}

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MUCH work has been done to explain the difference in the behavior of the serum bilirubin of hemolytic jaundice and that of obstructive jaundice with the van den Bergh reaction.

In general the attempt has been made to show that the two types of reactions could be explained by differences in the chemical or physical state of the bilirubin in the two types of jaundice. Van den Bergh¹ himself proposed these two possibilities. As to chemical differences, van den Bergh² and Andrewes³ have shown that the serum bilirubin giving the direct reaction oxidizes more easily than the serum bilirubin giving the indirect reaction. Collinson and Fowweather⁴ believe from their work that the bilirubin giving the "direct" reaction is probably an ammonium salt of bilirubin, while the "indirect" type of bilirubin is the free acid. Barron⁵ was unable, however, to confirm their experiments.

Regarding differences in the physical states of the two types of bilirubin, van den Bergh² found that the "direct" bilirubin was more easily adsorbed by protein precipitated by alcohol than is the case with the "indirect" bilirubin. Grunenberg⁶ has reported differences in the chloroform solubilities of the bilirubin occurring in the two types of jaundice. Hoover and Blankenhorn⁷ and Leschke⁸ have reported that the bilirubin of obstructive jaundice differed from that of hemolytic jaundice in being readily dialyzable through collodion membranes. Gregory and Andersch⁹ have reported experiments which show that neither the serum bilirubin of obstructive jaundice nor the bilirubin which has passed through the liver cells (gall bladder bile) or through the kidney is filtrable or dialyzable through collodion membranes which will not permit the passage of protein.

Working with xanthochromic spinal fluid, Vaughan and Hubbard¹⁰ concluded that the type of reaction depends upon the quantitative relationship between the pigment and protein. They found with material of low protein concentration that when the bilirubin exceeded 0.3 mg. per cent the van den Bergh reaction became direct. They believe, therefore, that it is unnecessary to consider that passage through the liver cells is the determining factor which changes the "indirect" to the "direct" type of bilirubin.

Some of our experiments have yielded results which we believe show that the quantity of the serum bilirubin plays no part in determining whether the bilirubin will give the direct or the indirect type of van den Bergh reaction.

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METHODS

Blood samples were obtained from the cords of 11 babies at birth. These bloods were analysed for serum bilirubin according to the Gibson and Goodrich modification¹¹ of the van den Bergh reaction. Most of the babies were observed for three days, and a urine specimen obtained three to five days after birth.

Gall bladder bile obtained at autopsy was filtered and diluted with pooled serum, to make a series of sera containing from 1.8 mg per cent (the diluting serum, van den Bergh indirect) to 12.5 mg per cent of bilirubin. These sera were analysed by the quantitative van den Bergh test as modified by Gibson and Goodrich.

TABLE I

BABY	CLINICAL JAUNDICE	VAN DEN BERGH MG %	TYPE OF VAN DEN BERGH	COLOR OF URINE	GIELIN TEST ON URINE
O	+	26.0	Indirect	Straw colored	Negative
G		9.6	Indirect	Straw colored	Negative
E		12.2	Indirect	Water clear	Negative
W	+	13.0	Indirect	Straw colored	Negative
L	++	28.8	Indirect	Straw colored	Negative
K	+	26.2	Indirect	Straw colored	Negative
S	+	22.2	Indirect	Straw colored	Negative
L		7.6	Indirect	Straw colored	Negative
M ₁		2.8	Indirect	Straw colored	Negative
W		11.4	Indirect	Straw colored	Negative
M		18.5	Indirect	Straw colored	Negative

TABLE II

SERUM DILUTION	VAN DEN BERGH MG %	TYPE OF VAN DEN BERGH
Pooled serum	1.8	Indirect
1	3.0	Direct
2	4.1	Direct
3	5.2	Direct
4	6.2	Direct
5	7.2	Direct
6	8.0	Direct
7	9.4	Direct
8	10.2	Direct
9	11.2	Direct
10	12.5	Direct

Sera were prepared as follows: a few drops of filtered bile were added to the pooled serum. This resulted in a serum which gave a direct van den Bergh of 1.5 mg per cent. Sera 1 to 10 were obtained by diluting this with the pooled serum with van den Bergh of 1.8 indirect.

RESULTS

Our results are listed in the accompanying tables. Reference to Table I shows that bilirubin of the serum of cord blood gives unusually high values for indirect van den Bergh reactions. With the exception of babies G, W, and K, whose cord blood serum gave a very faint trace of color with the "direct" procedure, all of the values obtained were of the "indirect" type.

In Table II is shown the results of subjecting serum dilutions of filtered bile to the van den Bergh test. All of these diluted and "artificially" jaundiced sera gave direct van den Berghs, with values ranging from 3.0 to 12.5 mg per cent.

A van den Bergh value as high as 28.8 mg. per cent "indirect" in the cord blood and as low as 3.0 mg. per cent "direct" in artificially jaundiced sera, allows one to conclude that the results of Vaughan and Hubbard on spinal fluid are not applicable to blood.

We did not estimate the serum proteins on the cord blood. Peters and Van Slyke,¹² quoting Bakwin and Rivkin¹³ and Mello-Leitao,¹⁴ state that the serum of infants "contains considerably less protein than that of adults." Although neither of the original papers dealt with cord blood of newborns, blood from babies as young as twelve days was analysed for protein.

Assuming that these low plasma proteins occur in cord blood of newborns, it invalidates further the conclusions of Vaughan and Hubbard so far as blood bilirubin is concerned. In as much as it is their conclusion that the greater the amount of bilirubin in proportion to the protein in solution, the more likely a direct van den Bergh reaction will result.

The question naturally arises as to why the van den Bergh reaction of 1.8 mg. per cent "indirect" of the pooled sera became "direct" after the addition of the gall bladder bile. The nature of the substance in bile responsible for this is being further investigated.

CONCLUSIONS

The quantity of bilirubin in serum is not the factor which determines whether it will give the "direct" or "indirect" van den Bergh reaction. It appears unlikely that the quantitative interrelationship between serum bilirubin and protein is the determining factor.

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ELECTROCARDIOGRAPHIC STUDIES DURING ANESTHESIA WITH INTRAVENOUS BARBITURATES*

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TOXIC effects from anesthetic drugs are dramatically displayed when the respiratory or circulatory functions are seriously disturbed. A number of procedures for promptly restoring these vital activities to normal have been advanced. The anesthetist is not without adequate methods to contend with respiratory arrest but is afforded little security with his efforts when confronted with severe cardiac disturbances. The inability to effect a favorable result for not a few of these patients has focused attention upon the causes of such complications. Consequently, the effects of various anesthetic agents on the heart have been the subject of many recent investigations.

Kurtz and his associates¹ reported an extensive clinical study of the electrocardiographic changes during anesthesia with most of the anesthetic agents in common use. Arrhythmias and other cardiac irregularities were not uncommon. Similar studies have been completed in other clinics,^{2, 3} but the changes during anesthesia from the intravenous use of soluble barbituric acid derivatives have not been included.

Gruber⁴ first presented the results from electrocardiographic studies with barbituric acid derivatives. In discussion at the annual Congress of Anesthetists, October, 1936, he reported preliminary observations which indicated that the thio derivatives of barbituric acid employed as anesthetic agents consistently produced cardiac irregularities in laboratory animals. These disturbances were accentuated if the thio barbiturates were combined with morphine. Barbiturates which did not contain a sulfhydryl radical produced no such cardiac disturbances. Since then Gruber has studied this phenomenon more extensively and has found a similar reaction in practically every instance for different species of laboratory animals. Kohn, working in the experimental division of the Abbott Laboratories, found no constant electrocardiographic changes in animals anesthetized with sodium thio pentobarbital.⁵ Reme and others recently reported electrocardiographic studies during peritoneal and evipan anesthesia in animal experiments.⁶ They found alterations in the electrocardiogram during anesthesia with these agents, but attributed the changes to anoxemia rather than to the anesthetic agents. Hafkesbrugg and MacCalmont⁷ studied electrocardiographic changes during anesthesia with nembutal, amytal, and barbital in the dog and cat. They observed no outstanding irregularities in cardiac rhythm or conduction.

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The increasing popularity of intravenous anesthesia with the barbituric acid derivatives, especially the thio derivatives, and the electrocardiographic changes observed in laboratory animals warranted a clinical study by means of the electrocardiogram while these drugs were employed.

The drugs used in this study were sodium thio-pentobarbital (pentothal or sodium thionembutal), sodium isoamyl ethyl thio-barbiturate (sodium thio-amytal),* and sodium N-methylcyclohexenyl-methyl barbituric acid (evipal soluble). The latter two were employed in 10 per cent aqueous solution. A 5 per cent aqueous solution of pentothal was used. There was a total of 17 anesthetics for 12 patients. Three of the patients were anesthetized at different times with different agents.

Patients were chosen at random from a general surgical ward and had only the routine physical examination and laboratory study. The surgical procedures were of a minor nature but required obtundation of pain (e.g., removal of a painful dressing or manipulation of arthritic extremities). Guedel's signs of anesthesia were followed for the classification of degrees of narcosis.

In eight cases preanesthetic medication with morphine sulfate $\frac{1}{8}$ to $\frac{1}{4}$ gr. (0.0075 to 0.015 gm.) or atropine $\frac{1}{150}$ to $\frac{1}{100}$ gr. (0.0004 to 0.0006 gm.) or a combination of these two drugs was given subcutaneously one hour before anesthesia. Ephedrine sulfate and adrenalin hydrochloride were given intravenously to effect (rise in systolic blood pressure and an elevation in pulse rate) at the height of the anesthesia in an attempt to sensitize the myocardium to any possible minimal effect of the anesthetic agent on the heart. Seven cases received no drugs other than those used to produce narcosis.

Studies were completed by securing a preliminary or normal electrocardiographic tracing, using the three leads. The barbiturate was then given intravenously until surgical anesthesia was obtained while Lead II was continually being recorded. Tracings of Lead II were completed for a short time after the height of anesthesia was reached and in some cases during the surgical procedure. All three leads of the electrocardiogram were taken during recovery until the patient had reached the status of rational response to questioning.

The cases in which pentothal was employed demonstrated by electrocardiographic tracings only a slight increased heart rate. There were no QRS or T-wave abnormalities.

CASE REPORTS

G. P., (Fig. 1), a fifty-two-year-old male. History and physical examination were essentially negative, except for local surgical condition (hemorrhoids). On admission, patient's blood pressure was 110/60, pulse rate 80, W.B.C. 9,500, R.B.C. 4,500,000, hemoglobin 85 per cent. Pentothal was given on two successive days to facilitate changing a painful posthemorrhoidectomy dressing. For the first anesthetic no preliminary medication was employed, but for the second, morphine sulfate $\frac{1}{4}$ gr. (0.015 gm.) was administered an hour before an

*Parke, Davis & Co., Detroit, Mich.

esthesia. An electrocardiogram, prior to anesthesia, revealed a right bundle branch block with a deep S wave in Lead I and a QRS of 0.12 second. No appreciable change occurred in the bundle branch block or the P-R interval during anesthesia on either occasion. The only recorded difference was a slight and temporary increase in pulse rate.

A. C. (Fig. 2), a white male, fifty years of age, studied one month after operation for relief of acute intestinal obstruction. There was a well functioning ileostomy. Patient's history

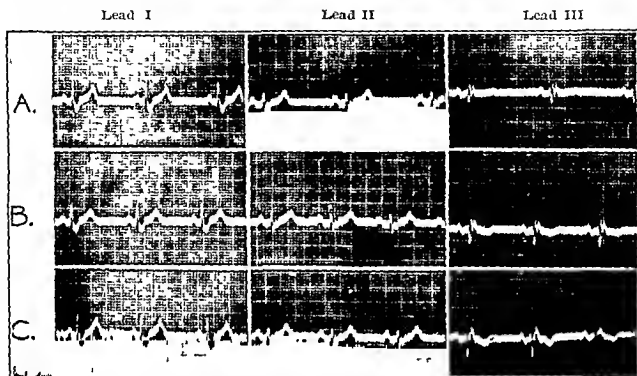


Fig. 1.

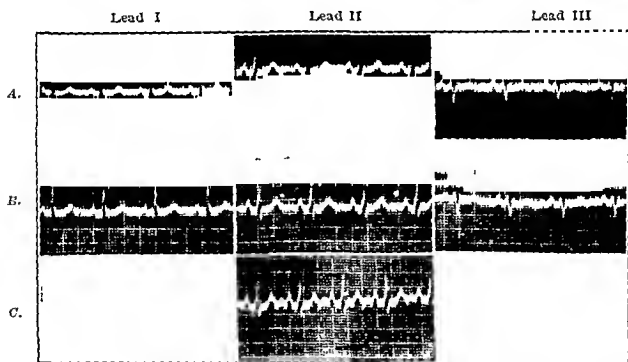


Fig. 2.

and physical condition essentially negative, except for the local surgical condition and a moderate secondary anemia. Blood pressure 140/70, pulse rate 88, temperature (rectal) 99.4° F, R.B.C. 3,500,000, W.B.C. 14,600, hemoglobin 80 per cent. One and one half hours prior to anesthesia, morphine sulfate 1/6 gr. (0.01 gm.) was given subcutaneously. The blood pressure was 110/60 and the pulse rate 70 immediately before anesthesia. Two hundred and fifty milligrams of pentothal were sufficient to induce surgical anesthesia. At the height of narcosis one minim of epinephrine hydrochloride (1/1000) in 1 c.c. of sterile saline was given intravenously. Within forty-five seconds the blood pressure increased from 110/70 to 180/90

and the pulse rate from 70 to 124. Five minutes later the blood pressure was 90/50 and the pulse rate 78. During the entire period of anesthesia, there were no changes in the electrocardiographic tracings other than an increase in the heart rate.

J. M. (Fig. 3), a fifty-year-old white male. History and physical examination essentially negative, except for a painful hypertrophic arthritis of the right wrist. Temperature was 99° F., pulse rate 80, blood pressure 150/90, R.B.C. 3,900,000, W.B.C. 10,500, hemoglobin 90 per cent. No premedication was given. At onset of anesthesia the blood pressure was 160/100, pulse rate 90. Six hundred milligrams pentothal were used to produce the desired anesthesia. This was followed immediately by the intravenous administration of one and one-half minim adrenalin (1/1000) in 1.5 c.c. of normal saline. One minute later, the blood pressure reached a maximum height of 195/105. The pulse rate was then 120. In eight minutes the blood pressure was 145/95 and the pulse rate 96. During this interval, the arthritic wrist was manipulated. Throughout the entire procedure the electrocardiogram demonstrated no change except an increased heart rate.

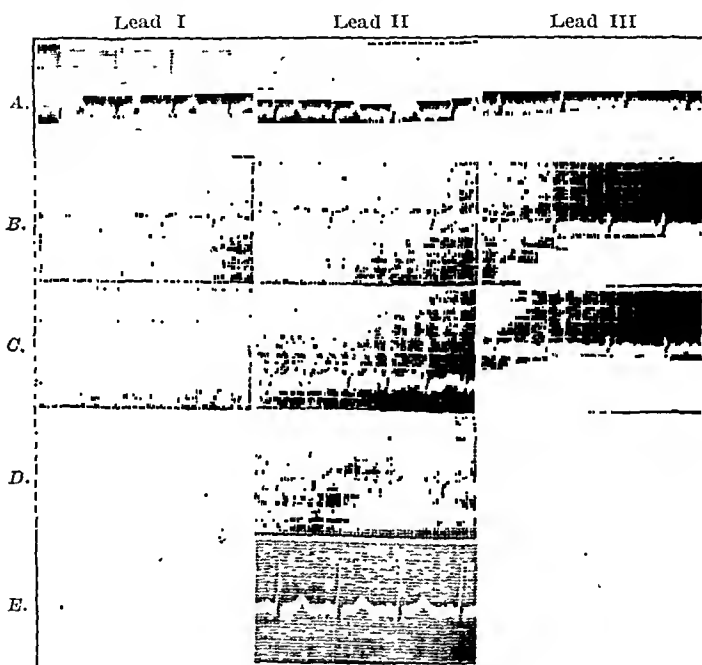


Fig. 3.

Eight electrocardiographic studies were completed during the intravenous administration of thio-ethamyl. The electrocardiograms revealed no changes in the QRS complex, P-R interval, or T-wave. There was noted only an increase in heart rate.

R. M. (Fig. 4), fifty-two-year-old white male. History and physical examination entirely negative, except for local surgical condition, cellulitis of the right foot, which had been incised and drained two days previously. Temperature 101° F., R.B.C. 4,200,000, W.B.C. 20,100. Blood pressure 160/100, and pulse rate 80 before anesthesia. No premedication was employed. Electrocardiograms before anesthesia revealed a regular sinus rhythm with ventricular premature contractions and coupling. The QRS was slurred in Leads II and III. Five hundred milligrams of thio-ethamyl were given intravenously to produce surgical anesthesia. Lead II of the tracing, taken during induction, showed a rate increased to 100, but the ventricular premature contraction and coupling remained unchanged for four minutes

during the anesthesia and then suddenly disappeared and a normal rhythm was observed during the remainder of the anesthesia and throughout the recovery period. There were no changes in the QRS or P-R interval.

M. M. (Fig. 5), colored female, aged thirty four years. History and physical examination were essentially negative, except for the local surgical condition. A human bite on the

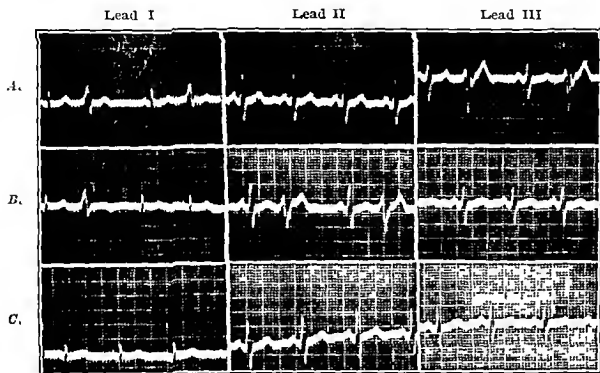


Fig. 4.

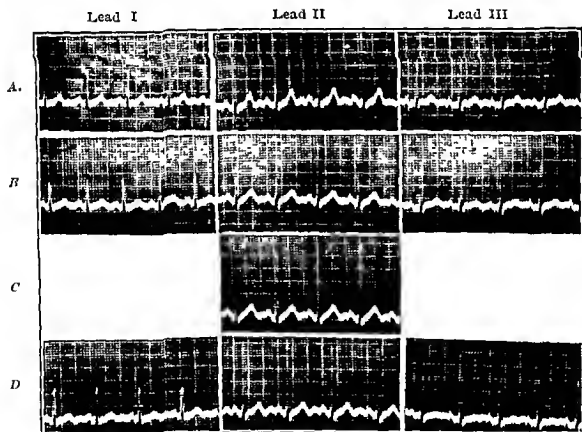


Fig. 5.

left thumb had been incised and drained one day before. Blood pressure was 125/82, pulse rate 84, temperature 98.4° F. No preliminary medication was given. Twelve hundred and fifty milligrams of thio ethamyl were required to produce surgical anesthesia. Blood pressure was then recorded 112/70, pulse rate 120. Twelve and one half milligrams of epinephrine sulfate in 5 c.c. of sterile water were injected intravenously. Within forty seconds there was an increase in blood pressure to 155/85. Three and one half minutes later the blood pressure

was at the original level. During this interval drains in the infected thumb were replaced. The electrocardiogram revealed no abnormalities at any time during the procedure except an early, transient tachycardia.

E. M. (Fig. 6), white male, aged fifty-two years. History of chronic alcoholism. Admission for cellulitis of the right forearm. Temperature 98.8° F., pulse rate 90, blood pressure 115/80. R.B.C. 3,730,000, W.B.C. 12,900, hemoglobin 65 per cent. Patient received subcutaneous morphine sulfate 1/6 gr. (0.01 gm.) and atropine 1/150 gr. (0.0004 gm.) one hour before anesthesia. Seven hundred milligrams of thio-ethamyl produced surgical anesthesia. Blood pressure was 110/75 and pulse rate 90 at this time. Twenty-five milligrams of ephedrine sulfate diluted with 10 c.c. of sterile water were given intravenously within two minutes. Blood pressure was then 140/85 and the pulse rate 100. Lead II of electrocardiogram taken during and after the procedure did not deviate from the normal.

In the same manner three patients were given evipal soluble intravenously to produce surgical narcosis. The first received no premedication, the second received morphine 1/6 gr. (0.01 gm.) as a preliminary sedative, the third morphine sulfate 1/6 gr. (0.01 gm.) and atropine 1/150 gr. (0.0004 gm.). Electrocardiographic tracings in these three cases were normal.

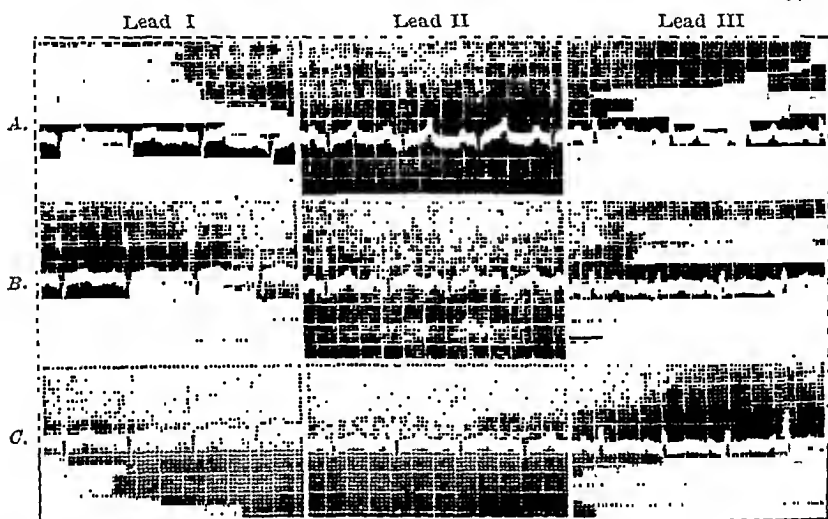


Fig. 6.

In every instance where atropine was employed as a preanesthetic drug in conjunction with the barbiturates, the return of the ventricular rate to normal was delayed.

SUMMARY

In seventeen clinical trials the electrocardiogram revealed no deviation from the normal in the QRS, P-R interval, or T-wave when either sodium thio-pentobarbital (pentothal), sodium isoamyl ethyl thio-barbiturate (sodium thio-ethamyl), or sodium N-methylcyclohexenyl-methyl barbituric acid (evipal soluble) was employed intravenously to produce surgical narcosis.

Preliminary medication with morphine sulfate, with atropine or with the two combined, affected the heart by prolonging an increased rate. Patients receiving atropine showed this effect constantly.

Epinephrine hydrochloride or ephedrine sulfate given intravenously in small amounts at the height of thio-barbiturate anesthesia did not precipitate cardiac irregularities.

Cardiac irregularities already present were not altered by the drugs used in this investigation

This investigation supported by a grant from Parke Davis & Co. Detroit Mich. was completed under the direction of Dr. Arthur C. DeGriff, Department of Therapeutics and Dr. E. A. Roventine, Department of Anesthesia

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THE INCIDENCE OF TUBERCULOSIS IN A NEW YORK CITY DIABETIC CLINIC*

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IN RECENT years there have been an increasing number of reports on the association of pulmonary tuberculosis and diabetes. The paper by Root¹ in 1934 reviews the literature so thoroughly that it seems unnecessary to repeat what he has already said. Since then a number of other articles have appeared on the subject.²⁻⁶ King⁷ has pointed out that, although tuberculosis complicating diabetes has decreased during the past fifty years, it is still approximately five times more frequent in diabetics than in the population as a whole. Most of the reports have come from tuberculosis sanatoriums. We have studied the occurrence of tuberculosis in the diabetic patients attending the Diabetic Clinic of the Third Medical Division (New York University), Bellevue Hospital, and the Diabetic Clinic of New York University College of Medicine. The total number of diabetics attending these clinics is, at present, 748. The observations were made over a period of seven years.

Of the 748 patients, 33 had active pulmonary tuberculosis, an incidence of 4.3 per cent. Table I gives the sex and age of these patients, the time of onset of the diabetes and the tuberculosis, and the diets and amounts of insulin needed. Thirteen of the patients were females, twenty one were males. The age at which the onset of tuberculosis occurred varied from nineteen to seventy nine years, seventeen were under fifty years of age. Excluding the

*From the Diabetic Clinics of the Third Medical Division (New York University), Bellevue Hospital and the New York University College of Medicine Clinic.

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one patient who was seventy-nine, which seems to us exceptional, the average age was 47.8 years. This is a somewhat higher age incidence for the onset of tuberculosis than occurs in the population as a whole. In 29 cases the onset of the diabetes preceded, as far as we could ascertain, any signs or symptoms of tuberculosis. In 3 cases the diagnosis was made simultaneously. The diets used in these patients were high in carbohydrate, moderate to high in fat,

TABLE I
CLINIC DIABETIC PATIENTS WITH TUBERCULOSIS

CASE NO.	SEX	AGE	DIAG- NOSIS OF DI-	DIAG- NOSIS OF	DIET C P F	DAILY IN- SULIN UNITS	CLASSI- FICA- TION OF TBC.	COURSE
			ABETES	TBC.				
1	F	31	1929	1928	150-70-140	20	III-C	Died, 1930
2	F	43	1921	1929	140-60-90	35	III-C	Died
3	F	19	1928	1929	125-60-130	15	II-C	Sanatorium
4	M	39	1927	1930	160-75-120	30	III-C	Died
5	F	33	1929	1930	150-65-115	27	III-B	Died
6	F	58	1917	1930	200-90-105	10	III-B	Oleothorax—doing well
7	M	62	1928	1930	180-70-85	15	III-C	Died—perforated gastric ulcer
8	M	62	1926	1930	150-64-100	10	II-B	No follow-up
9	F	48	1924	1930	130-64-100	15	III-C	Died
10	M	48	1927	1930	150-65-120	10	III-B	Died
11	F	23	1929	1931	280-77-195	95	III-C	Died
12	M	62	1930	1931	150-60-100	40	III-C	Died
13	M	56	1926	1932	215-70-100	48	I-A	No follow-up
14	M	19	1929	1933	200-65-85	120	II-B	No follow-up
15	M	40	1932	1933	150-70-85	38	III-C	Died
16	M	51	1930	1933	350-80-120	95	III-B	Prognosis poor
17	M	40	1930	1934	250-90-110	75	III-B	Prognosis poor
18	F	48	1933	1934	180-70-85	20	II-B	Sanatorium
19	M	65	1933	1934	200-75-65	10	III-B	Prognosis poor
20	M	56	1931	1934	300-90-100	18	III-B	No follow-up
21	F	48	1917	1930	150-65-85	25	II-A	Arrested
22	F	52	1928	1934	200-65-85	35	III-B	Prognosis poor
23	F	61	1931	1934	150-65-85	35	III-B	Died
24	M	46	1935	1935	250-65-85	55	III-B	Prognosis good
25	F	26	1929	1935	300-65-130	70	III-C	Died
26	M	51	1930	1923	200-65-120	40	II-A	Arrested
27	M	69	1933	1936	150-65-85	12	I-A	No follow-up
28	M	46	1934	1936	325-80-85	105	III-B	Sanatorium
29	M	79	1934	1936	200-70-85	20	III-C	Died
30	M	64	1927	1936	180-70-85	60	II-A	Arrested
31	M	58	1936	1936	180-65-85	50	I-A	Arrested
32	F	46	1934	1935	250-65-85	100	III-A	Arrested
33	M	32	1931	1931	250-70-100	65	III-B	Prognosis poor

and with a protein intake of 65 to 85 grams. All but one patient required insulin, and twenty-two of them required more than thirty units daily.

On admission the tuberculosis classification, according to the National Tuberculosis Association criteria, was as follows:

Stage I	3 cases	9.0 per cent
Stage II	7 cases	21.0 per cent
Stage III	23 cases	68.9 per cent

Treatment of the Tuberculosis.—Ten of the patients received pneumothorax. Two had, in addition, pneumolyses which were not successful. In one case there was hemoptysis which could not be controlled by pneumothorax or

phlebotomy, and the patient finally died of a bronchogenic spread. Five of the patients during their hospital period, left against advice, and we have been unable to obtain any further information about them.

Course—Of the 33 patients observed, 13 have died since they developed tuberculosis. Ten no longer attend the clinic, but as far as we know are still alive. Of the remaining 10, 5 are now attending the diabetic clinic, having been treated successfully for their tuberculosis. The remaining 5 are at present in sanatoriums. Three of the five patients attending the clinic are considered inactive.

Treatment of the Diabetes—The combined incidence of these two diseases is interesting because of the effects of diabetes on the general nutrition of the individual and because of the importance of nutrition in tuberculosis. Diabetes, disturbing as it does not only the carbohydrate, protein, and fat metabolism of the individual, but also the metabolism of calcium, phosphorus, and certain of the vitamins, would naturally influence the course of a chronic infectious disease, such as tuberculosis. In considering the diabetic with tuberculosis, particular attention should be paid to the general state of nutrition of the individual and effort made not only to control the diabetes, but to provide the patient with a diet adequate in every respect. It is our policy in treating the diabetic patient with tuberculosis to give a diet which is ample in calories (2,500 to 3,000). The protein content averages from 65 to 85 grams, the carbohydrate from 150 to 300 grams, and the rest of the diet is made up with fat. In order to assure an adequate amount of vitamin A, part of this fat is given in the form of cod liver oil. In view of the high blood carotene that has been reported in diabetes,^{8, 9} particularly when fed carotene, it seems wiser to give vitamin A as such rather than as its precursor.

As the tuberculous diabetic is apt to be a severe diabetic, we have found it advisable to feed the individual four times in twenty-four hours, rather than the usual three meals, as the latter leaves the patient with no outside source of energy for a period of fourteen hours, during which time he would be forced to burn his body stores. Insulin is given with each feeding in amounts sufficient to keep the patient approximately sugar free, and always acetone free. We advise the patients to eat at 8 A. M., 1 P. M., 6 P. M., and 11 P. M.

It is equally important that the intake of fluid and salt should be adequate. Diabetics become dehydrated very readily, particularly in the presence of an infection. We, therefore, advise the patients to drink ample amounts of water and to take a slight excess of salt. Normally an individual takes in approximately 4 grams of sodium chloride as such daily. In the severe tuberculous diabetic this should be increased to 6 grams a day or more if necessary. An adequate intake of calcium is assured by having the patient take at least three glasses of milk daily.

EXAMPLES OF CLINIC CASES

X-rays are shown on 3 of the clinic cases followed. The first of these, Case 14, a nineteen-year-old boy, four years after developing diabetes was found to have minimal tuberculous infiltration when admitted to the hospital. Absolute rest was advised, but as the

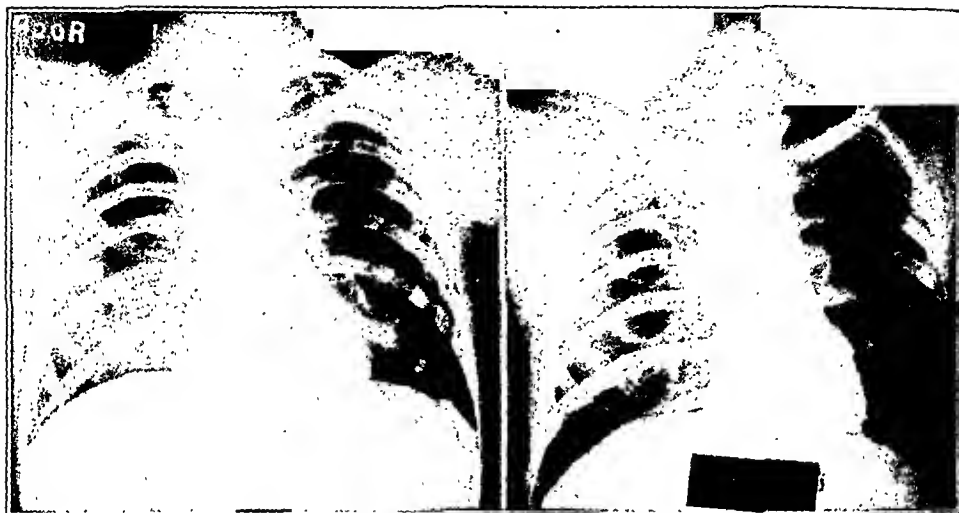


Fig. 1.—Case 14.

X-ray taken Jan. 9, 1933.

Early x-ray showing minimal degree of tuberculous infiltration at right apex.

X-ray taken Jan. 29, 1933.

Extension of tuberculous process to the third right anterior rib area.



Fig. 2.—Case 8.

X-ray taken Sept. 11, 1935.

Widespread cavitation in upper third of right lung field with bronchiogenic spread to the base. Bronchiogenic spread in left midlung field with a small cavity.

X-ray taken Feb. 11, 1936.

Right hydropneumothorax with marked displacement of heart and mediastinum to the left. (Spontaneous bronchopleural fistula present on this side.) Widespread tuberculous bronchiogenic spread throughout left side.

patient was unruly he left the hospital against advice. A later x ray shows a spread of the lesion, although the sputum was still negative. He was finally persuaded to go away to a sanatorium.

CASE 8—A female, aged twenty six years, with a history of diabetes of six years' duration. She had never cooperated in the treatment of her diabetes and had been admitted frequently in diabetic ketosis. After an absence from the clinic of four months she was admitted to the hospital in profound ketosis and at this time was found to have advanced tuberculosis. Treatment consisted of pneumothorax on the right, although the lesion was bilateral. Pneumolysis was done but was unsuccessful, and the patient finally died.

CASE 24—A forty six year old male developed diabetes and tuberculosis apparently at the same time. When he was first seen, there was a large cavity in the left midlung field. Pneumothorax was given and the patient's course was satisfactory, the sputum becoming negative. He now attends the diabetic clinic and is receiving pneumothorax.

Hospital Group (Table II)—During the period of time that these patients were observed in the clinics, patients with diabetes and tuberculosis, who had



Fig 2—Case 24

X ray taken Dec 30 1935
Huge cavity in upper third of lung field with
bronchogenic dissemination from apex to
fourth anterior rib

X ray taken March 18 1936
Adequate pneumothorax collapse on the left.

never been previously treated in the clinic, were admitted to the Third Medical Division (New York University), Bellevue Hospital. These patients were either unaware of their diabetes, or the diagnosis having been made, had not bothered with any treatment. The total number of such patients from 1929 to 1936 was 21. The total number of diabetic patients admitted to the Third Medical Division during this same time was 986, giving a 2.5 per cent incidence of tuberculosis in the group of diabetic patients. All were in Stage III, far advanced tuberculosis, when admitted. The average age was 48.3 years. All but 3 were over thirty years of age. In every case there was knowledge of the diabetes before the tuberculosis. Six of the patients became aware of the existence of both diseases when they entered the hospital. In 11 patients, although diabetic symptoms were present before admission, treatment for

both diseases began only after hospitalization. Of the 21 patients, 14 have died, and 2 are still in sanatoriums; one, although quite sick, attends the clinic. There are no follow-up records on the other four.

TABLE II
NONCLINIC DIABETIC PATIENTS WITH TUBERCULOSIS ADMITTED TO THE HOSPITAL

NAME	SEX	AGE	DIA- BETES DIAG- NOSED	TBC. DIAG- NOSED	DIET C P F	DAILY IN- SULIN UNITS	TBC. CLASSI- FICA- TION	COURSE
A. K.	M	50	1925	1929	100-60- 90	0	III-C	Died
E. M.	F	40	1929	1929	160-70-140	60	II-C	Died
S. B.	M	27	1929	1930	190-90-160	42	III-C	Probably dead
P. G.	M	53	1925	1930	175-70-120	10	III-C	Died
J. M.	M	52	1920	1930	130-64-100	0	III-B	No follow-up
S. P.	F	60	1930	1932	Unable to eat		III-C	Died
J. F.	M	43	1929	1930	150-64-100	70	III-A	Prognosis poor
R. C.	M	48	1930	1932	200-65- 85	38	III-B	Died
A. H.	F	29	1928	1932	200-60- 85	13	III-C	Died
A. H.	F	48	1931	1931	150-64-100	27	III-C	Probably dead
E. M.	M	32	1932	1932	260-70-130	58	III-B	No follow-up
E. O.	F	65	1931	1932	Ketosis regime		III-C	Died
J. A.	M	43	1931	1933	230-70-155	48	III-C	Died
C. W.	M	68	1928	1933	Unable to eat		III-C	Died
M. S.	F	54	1926	1933	200-65- 85	25	III-C	Died
S. T.	M	33	1933	1933	250-65- 85	0	III-B	Sanatorium
J. F.	M	61	1929	1934	Unable to eat		III-C	Died
P. P.	M	38	1935	1935	180-70- 85	30	III-B	Prognosis bad
L. S.	M	46	1931	1935	200-75- 85	15	III-C	Died
J. S.	M	69	1933	1935	250-75- 85	0	III-C	Died
J. R.	M	58	1936	1936	250-65- 85	60	III-C	Died

Treatment and Course of the Tuberculosis in This Group.—Pneumothorax was tried on 6 patients, all unsuccessful because of adhesions and of the progress of the disease. Two of them left against advice and were not adequately followed. The remainder were given bed rest only. Since every case was in the far-advanced stage when they first entered, it is only natural to expect that the majority by this time have died.

DISCUSSION

Although the prognosis in diabetes and tuberculous is still far from encouraging, the outlook need not be hopeless. This is borne out by the difference in the severity of the tuberculosis in the group of patients who were never properly treated for their diabetes and were admitted to the hospital for both diabetes and tuberculosis, as contrasted with the diabetic patient treated in the clinic who received adequate treatment early for both diseases. Several facts account for the better prognosis in the clinic group. Attending as they do a chronic disease clinic at regular intervals and being under medical supervision, the diagnosis is usually made early, which makes it possible to give the patient better care for both diseases. Additional evidence of this fact is that several of the patients in the clinic group who died were patients who did not attend the diabetic clinic regularly and who had been admitted to the hospital several times in diabetic ketosis, due to lack of adherence to diet and insulin. Other observers of these combined diseases have pointed out the fact that acidosis influences the prognosis unfavorably, as it increases the loss of

calcium from the body, causes dehydration, blood concentration and loss of base. Furthermore, the uncontrolled diabetic is in a poor state of nutrition, which would further reduce his resistance to infection.

It is in the clinic group that there is the greatest hope for the prevention of advanced tuberculosis. If the diabetic patient is manageable, it should be possible to have routine x-rays of the chest every six months. For the past three years, we have tried to x-ray routinely every diabetic patient attending the clinic, and up to date have been successful in x-raying 80 per cent of the cases. If this is done, the tuberculous involvement may be discovered in its minimal stages and adequate treatment may arrest the disease.

The same general principles that are followed in the treatment of the nondiabetic tuberculous patients are advocated for the diabetic patient. It is, however, essential in the diabetic that the diabetes be well controlled at all times. With late cases in which the diagnosis of tuberculosis is made when the disease is advanced, the prognosis, as our experience indicates, is hopeless even though all the modern methods of collapse therapy, including pneumothorax and pneumolysis, are utilized.

SUMMARY AND CONCLUSIONS

In two active diabetic clinics with a total registration of 748 patients, pulmonary tuberculosis was present in 45 per cent of the cases. Of the patients with pulmonary tuberculosis, 13 died during the period of observation, which covered a period of seven years.

The treatment of diabetes complicating tuberculosis is outlined, and the treatment of the tuberculosis in these cases is given.

Attention is again drawn to the importance of early diagnosis in these cases and of careful control of the diabetes.

We wish to acknowledge the assistance in this study of our associates in the Clinic: Doctors Hoch, Hansman, Lovelock, McGoe, and Crandell.

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DETECTION OF CIRRHOSIS AND OTHER DISEASES OF THE LIVER BY LABORATORY TESTS*

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LABORATORY tests for the diagnosis and study of liver diseases are numerous but skepticism prevails concerning their reliability. The hesitancy with which such tests are utilized seems unjustified if one may accept the conclusions of numerous investigations. Although it is true that many procedures advocated have been found subsequently to have little merit, the value of others has been established. Among the newer laboratory aids that have been proposed for the study or detection of liver disease, the Takata-Ara test, the fractionation of serum proteins, and the determination of esterified and free cholesterol have not been studied as extensively or correlated as completely with other tests as is desirable for evaluation. The present investigation is an appraisal of the accuracy of these procedures as used in a large general hospital, compared with estimation of icterus index and bromsulfalein retention under similar conditions. The records of over 250 patients in whom bedside examination demonstrated or suggested liver involvement were studied. In 228 patients it was possible with reasonable certainty to establish or exclude the presence of liver disease, and the data have been analyzed with the object of correlating the results of laboratory tests with clinical or pathologic findings.

The series included decompensated cirrhosis (57 cases), compensated cirrhosis (27 cases), acute diffuse parenchymal degeneration including "hepatitis" due to chemical or bacterial toxins, and so-called catarrhal jaundice (19 cases), chronic cholecystitis (27 cases), obstructive cholelithiasis (8 cases), hepatic neoplasm (17 cases), obstructive neoplasm (4 cases), chronic congestive heart failure (17 cases), and occasional cases of amyloidosis, fatty degeneration, tuberculous hepatitis, hemachromatosis, and syphilitic hepatitis. Liver disease was excluded in 38 cases. In certain groups data are insufficient to permit evaluation of the results of tests, but the findings have been tabulated because of their bearing on the interpretation of results in the larger groups.

In this investigation the Takata-Ara reaction was done by the modified technique described by Heath and King,⁷ serum albumin and globulin fractionation by Howe's procedure,⁸ using the macro-Kjeldahl method for nitrogen determination, icterus index according to Meulengracht,¹¹ and the total and esterified cholesterol by means of differences in rate of the Liebermann-Burchard reaction as described by Reinhold.¹² Our studies of serum cholesterol fractionation, however, are not complete, and results are reported only in cirrhosis. The qualitative van den Bergh reaction has been utilized solely

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as a test for bilirubinemia and findings are not presented. Galactose tolerance tests were done at times but failed to give sufficient information to warrant further study.

The Takata Ara reaction as a test for hepatic dysfunction has been studied intensively during recent years. Widespread interest has been created by the somewhat spectacular claim of specificity in the diagnosis of cirrhosis. Staub and Jezler first described the use of the test in diagnosis of liver disease, Hafstrom's⁶ monograph includes a review of the literature, and Kirk's⁹ paper summarizes recent work.

The occurrence of the various types of the Takata Ara reaction in cirrhosis as shown in Table I agrees closely with similar percentages calculated from the data of Heath and King. However, it is clear that reactions grouped as "suspicious" (questionable flocculent precipitate) had little diagnostic

TABLE I
TAKATA ARA REACTION

	STRONGLY POSITIVE	POSITIVE	WEAKLY POSITIVE	SUSPICIOUS	NEGATIVE
NUMBER OF PATIENTS					
Cirrhosis	24	33	4	2	9
Other parenchymatous liver disease	5	10	4	3	10
Parenchymatous liver dis- ease excluded	2	6	1	3	34
Total	31	49	9	8	53
Per cent having cirrhosis	78	67	44	25	17

significance, and that the accuracy of the test has been improved by considering these questionable reactions negative (as has been done in Table II). It should be noted that various authors differ in defining positive reactions and that results are not strictly comparable. We believe, however, that the technique of Heath and King employed by us has a higher precision in diagnosis of cirrhosis than that described by Crane,⁴ since at times in the presence of cirrhosis, precipitates were obtained only in small amounts in either or both dilutions of 1:32 and 1:64, reactions that are negative by the criteria of Crane.

Positive Takata Ara reactions were obtained in 45 of 49 patients having decompensated cirrhosis, and in 16 of 23 having cirrhosis without ascites. Positive tests were encountered also in other conditions associated with diffuse involvement of the liver: neoplasm, acute parenchymal degeneration, amyloidosis, fatty degeneration, and cholelithiasis of long duration. The reaction was positive in 3 of 14 cases of chronic congestive heart failure, and in 4 (subacute nephritis 2, pulmonary tuberculosis, lung abscess) in which liver disease was suspected and subsequently excluded.*

Change from a positive to a negative Takata Ara reaction may occur with regression of the causative disease. This has been observed to coincide with or follow clinical improvement, not only in hepatitis and similar acute conditions but also in patients with unmistakable evidence of cirrhosis.

*This series includes only tests done because of a clinical suspicion of hepatic disease. The Takata Ara reaction may be positive in a variety of extrahepatic diseases associated with serum protein changes, notably lobar pneumonia, tuberculosis, hyperthyroidism, multiple myeloma, subacute and chronic nephritis and nephrosis (see Bowman and Bray²¹).

TABLE II
SUMMARIZED COMPARISON OF TESTS

DIAGNOSIS	ICTERUS INDEX	BROMSULFALMIN RETENTION PER CENT			TAKATA-ARA	SERUM ALBUMIN PER CENT		SERUM GLOBULIN PER CENT		ALBUMIN-GLOBULIN RATIO						
		3-9	10-220	0-5.9		6-9.9	10-100	NEG.	WEAK POS.		1.5-3.8	3.9-5.2	1.7-3.1	3.2-5.8	0.27-1.3	1.3-2.2
NUMBER OF CASES																
Cirrhosis with ascites	Neer. Clin.	1	15	-	-	8	2	-	16	12	-	1	11	11	1	
		3	24	2 ^a	-	11	2	2	27	23	1	9	15	23	1	
Cirrhosis without ascites	Neer. Clin.	2	5	1	-	1	3	-	4	1	1	1	1	1	1	
		5	6	1	2	4	4*	2	10	7	1	4	4	5	3	
Hemachromatosis	Biopsy	1	-	-	-	1	-	1	-	-	-	-	-	-	-	
Neoplasm (obstructive)	Neer. Clin.	-	3	-	-	-	1	-	-	1	-	1	-	1	-	
		-	1	-	-	-	1	-	-	-	-	-	-	-	-	
Neoplasm (parenchymatous)	Neer. Clin.	3	6	2	-	2	3	2	2	1	1	2	-	1	1	
		2	6	1	1	2	2	-	3	2	-	1	1	2	-	
Cholecystitis	Neer. Clin.	-	2	1	-	1	-	-	-	-	-	-	-	-	-	
		6	8	11	6	7	5	-	-	1	3	3	1	1	3	
Cholelithiasis	Neer. Clin.	-	3	-	1	3	1	-	2	1	-	-	1	1	-	
		-	5	-	1	-	-	-	2	-	-	-	-	-	-	
Acute diffuse parenchymal degeneration	Neer. Clin.	-	2	-	-	-	-	-	1	1	-	-	1	1	-	
		-	17	-	1	4	6	-	3	3	-	1	2	3	-	
Fatty degeneration	Neer. Clin.	-	2	1	-	-	1	-	2	1	-	-	1	1	-	
		-	-	-	-	2	-	-	-	1	1	1	-	-	1	
Tuberculous hepatitis	Neer. Clin.	1	1	-	-	-	-	-	1	2	-	-	2	2	-	
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Syphilitic hepatitis	Biopsy Clin.	1	-	-	-	1	-	-	1	1	-	-	1	1	-	
		-	1	-	-	1	-	-	1	1	-	-	1	1	-	
Amyloidosis	Neer. Clin.	-	-	-	-	1	1	1	1	1	-	-	1	1	-	
		-	-	-	-	1	-	1	-	-	-	-	-	-	-	
Congestive heart failure	Neer. Clin.	-	2	-	-	2	2	-	-	1	-	-	1	1	-	
		1	3	1	2	4	9	-	3	3	2	4	1	2	3	
Liver disease excluded	Neer. Clin.	2	-	3	-	-	1	-	1	2	2	-	2	2	-	
		7	2	10	1	2	17	1	1	7	4	7	4	6	5	

*One subsequently positive.

Since various authors have shown that the Takata Ara reaction is closely related to abnormalities of the serum proteins, it seemed desirable to ascertain whether fractionation of the proteins might not prove to be a more reliable test. The prevalence of abnormally altered concentrations of albumin and globulin in the presence of diffuse involvement of the liver as shown in Table II is in accord with other reports.¹ In 58 of 72 patients with liver disease (of whom 46 had cirrhosis) albumin globulin ratios were less than 1.3. Sixty-two of this number had less than the minimum normal concentration of serum albumin, while 44 showed a rise in globulin above the upper limit of normal. Loss of albumin was usually compensated wholly or in part by increased globulin, so that serum total protein was infrequently abnormal. However, in the presence of malignancy or other wasting disease, low total proteins were characteristic. Here change was chiefly in the albumin fraction.

Almost every patient with decompensated cirrhosis showed abnormally low ratio of albumin to globulin. Likewise, only one patient showed an albumin concentration within the normal limits. Elevation of serum globulin seemed to be more prevalent in the more severe and terminal cases of cirrhosis. In general, alteration in serum proteins occurred less frequently in compensated cirrhosis. The incidence of abnormal albumin globulin ratio in all stages of cirrhosis was almost identical with that of positive Takata Ara reactions. However, in 3 patients abnormally low ratios were associated with negative Takata Ara reactions, while 6 with normal ratios gave positive Takata Ara reactions. Again, 6 patients with inversion of the albumin globulin ratio caused by renal disease gave negative Takata Ara tests, although positive tests have been observed in others with renal disease. Similar findings have been reported by other workers.^{15-20, 22} It is apparent that factors other than the ratio of albumin to globulin influence the characteristic flocculation of the Takata Ara reaction.

Serum cholesterol fractionation has been used in the investigation of liver disease since the demonstration by Thanuhausen and Schaber¹⁹ that esterified cholesterol decreased to low values when widespread injury to the liver occurred. Changes occurring in cirrhosis have been investigated by Epstein and Greenspan² and Adler and Lemmel.³ In the present study total and esterified cholesterol of serum was determined in one or more specimens from 48 patients with cirrhosis.

While the concentration of total serum cholesterol with a single exception remained within the limits of normal (found to be 100 to 350 mg per 100 cc), low normal values appeared with increased frequency. Concentrations of cholesterol combined as esters were often below normal in the presence of icterus (Table III). Cholesterol ester values in patients with cirrhosis and jaundice who died were compared with values obtained in those who were similarly jaundiced (icterus index over 15) but recovered. No significant difference was found.

The correlation of these newer tests with the widely used bromsulfalein and icterus index determinations is important. Following the introduction of bromsulfalein by Rosenthal and White,¹⁴ various workers³ demonstrated that retention occurred frequently in the absence of jaundice and that the

test was superior to most for the detection of cirrhosis. Originally 2 mg. of dye per kilogram of body weight was recommended, but later Greene advocated increasing the dosage of dye to 5 mg. The larger amount has been used in these studies. It appears that the proportion of the injected dye that is retained by normal individuals when 5 mg. per kilogram are administered has not been established with certainty, for various authors have regarded values ranging from 4 to 10 per cent retention at thirty minutes as normal. Although Soffer¹⁷ states that at the end of one-half hour never more than a trace of the dye has been found to be present in normal individuals, in a subsequent publication¹⁸ he regards 10 per cent retention at thirty minutes as the dividing limit. To clarify this detail, retention of the dye has been measured in 8 normal individuals. Between 0 and 6 per cent of the administered bromsulfalein remained in blood serum taken thirty minutes after the injection. One individual showed no retention, one showed 2 per cent, three showed 3 per cent, and one each 4, 5, and 6 per cent. These results suggest that the average amount of dye normally retained is between 3 and 4 per cent.

TABLE III

RELATIONSHIP OF TOTAL AND ESTERIFIED SERUM CHOLESTEROL TO ICTERUS INDEX IN CIRRHOSIS

		TOTAL CHOLESTEROL			ESTERIFIED CHOLESTEROL		
		MG. PER 100			C.C. SERUM		
		95-150	150-250	250-350	8-50	50-70	70-100
NUMBER OF PATIENTS							
Icterus index	Less than 15	7	8	3	2	6	10
	15 and over	12	23	7	15	8	19

Confirmation was given by tests done on 16 schizophrenic patients without apparent somatic disease. Three patients showed no retention, three showed 3 and 4 per cent respectively, 4 showed 5 per cent, and one each 6, 7, and 8 per cent. Because of their occasional occurrence in normal individuals, values between 6 and 9.9 per cent have been grouped separately in Table II. However, we believe that nearly all figures within this range are indicative of impaired excretory function.

In cirrhosis with ascites, the bromsulfalein test indicated distinctly abnormal liver function in 19 of 21 patients. One of the two negative tests was repeated several weeks later and definite retention found. In compensated cirrhosis, 6 tests were positive, two between 6 and 10 per cent, and therefore suspicious, and two were negative. In the latter two, the Takata-Ara reaction was positive. On the other hand, dye retention occurred in 5 patients with cirrhosis in whom the Takata-Ara reaction was negative.

Bromsulfalein injection detected hepatic insufficiency resulting from neoplastic metastasis to the liver in 3 patients without jaundice, but failed in 3 with icterus indices of 6, 8, and 14. Retention occurred with noteworthy frequency in cholecystitis and in chronic congestive heart failure. Occasionally the bromsulfalein test was positive in the absence of liver disease. Of 17 patients without demonstrable disease of the liver, 3 showed retention exceeding 6 per cent, the figures being 9, 12, and 14 per cent.

The use of bromsulfalein was restricted to patients with little or no jaundice. Marked retention has been found to occur invariably in the presence of jaundice, and the difficulty of determining accurately the degree of retention in highly icteric sera impairs the value of the test for comparative purposes. The choleric action of bromsulfalein affords another objection to its use in patients with deep jaundice of uncertain etiology.

The estimation of icterus index is a helpful procedure, for abnormal concentration of bile pigment in the blood may exist in the absence of clinical jaundice. Although Meulengracht, who introduced the test, regarded 10 as the upper limit of normal, 4 to 6 is commonly, yet erroneously, accepted as the normal range. In this laboratory, values as high as 9 are encountered in healthy individuals. Values above 9 occurred in more than 90 per cent of patients with decompensated cirrhosis, but in only 60 per cent of those without ascites. Ten patients with cirrhosis had normal icterus indices and positive Takata Aia reactions, frequently the latter was strongly positive with only minor elevation of the icterus index. The marked elevation of the icterus index encountered at times in congestive heart failure is noteworthy. Two patients without demonstrable disease of the liver had icterus indices of 10 and 11. We continue to find a relationship between icterus index values and type of qualitative van den Bergh reaction, in agreement with the observations described in an earlier report¹⁰.

DISCUSSION

Our data indicate that the tests investigated are sufficiently accurate to make them effective supplements to clinical examination for diagnosis of liver disease. The results demonstrate anew that none of the tests appraised can be relied upon to give conclusive evidence of minimal lesions of the liver and therefore that several tests should be employed where doubt exists.

Frequently the results of these tests have merely substantiated diagnoses clinically obvious. Nevertheless, their special value has been demonstrated on several occasions when clinical findings were inconclusive. This has been true particularly when bromsulfalein retention or the Takata Aia test have given the only tangible evidence of liver involvement before development of ascites and other manifestations of the advanced stage of the disease. Information secured by the application of these tests has been useful also in differentiating hepatic from other abdominal tumors, and in indicating the cause of hematemesis to be varices due to cirrhosis rather than ulcer.

The bromsulfalein test was somewhat more sensitive and accurate than the others studied. Although a slightly different procedure was used, our conclusions in this respect agree with those of Magath¹⁰. It can be used most effectively when jaundice is absent or slight, with the serum bilirubin concentration markedly elevated, the bromsulfalein test is superfluous and unsatisfactory. Rarely failing to detect liver damage when this was demonstrated by other methods, the dye test was frequently the only one to reveal hepatic disease. Retention in extrahepatic diseases noted by Robertson, Swalm and Konzelmann¹³ occasionally has been encountered. It is questionable whether involvement of the liver sufficient to cause dye retention could

not have existed in these patients despite clinical evidence to the contrary. If 10 per cent retention at thirty minutes is regarded as the limit of normal when 5 mg. per kilogram are injected, the accuracy of the test is diminished somewhat. Undoubtedly, the use of this limit explains to some extent the unfavorable conclusions of Soffer and Paulson¹⁸ concerning the bromsulfalein test as compared with the bilirubin retention test. As pointed out, only 4 of 24 controls in our series showed retention in the 6 to 9.9 per cent range. We believe, therefore, that retention in the borderline region between 6 and 9.9 per cent should be regarded as a probable indication of liver insufficiency.

The Takata-Ara reaction, which is easily performed, may be advantageously used either as a preliminary or as a confirmatory test. In the detection of early cirrhosis, it is somewhat less sensitive than the dye test. Positive in most cases of advanced cirrhosis, it is often positive as well in other conditions extensively involving the liver, and cannot be relied upon to differentiate these conditions from cirrhosis. Crane's⁴ conclusion that the chief merit of the test lay in its ability to distinguish cirrhosis from tumors was not substantiated by our findings, for positive reactions were frequent (although less common than in cirrhosis) in neoplastic disease of the liver. Although lacking specificity for hepatic disease, positive reactions were misleading only in a tuberculous patient who was thought to have an incidental cirrhosis; necropsy revealed no lesion of the liver. Two cases of subacute nephritis with anasarca considered to be possible cirrhosis gave positive Takata-Ara reactions, but the correct diagnosis was established without difficulty. A common and sometimes perplexing problem is that of differentiating the ascites of cirrhosis from that of congestive heart failure. Here, where bromsulfalein retention is, as a rule, marked and may be interpreted erroneously as indicating cirrhosis, the Takata-Ara test is less often misleading; a positive reaction is suggestive of cirrhosis.

The frequency with which serum albumin was lowered and globulin increased in diseases of the liver is especially worthy of note. Although the Takata-Ara test seemed a more dependable guide to the recognition of liver lesions, nevertheless diminution or inversion of the albumin-globulin ratio should be recognized as a common manifestation of diffuse liver disease.

The icterus index was infrequently elevated in the absence of liver injury. Chronic liver disease, however, was often revealed by bromsulfalein retention, a positive Takata-Ara reaction, and inversion of the albumin-globulin ratio with the icterus index normal.

The concentration of total cholesterol in serum was not markedly altered in cirrhosis, although low values were common. Esterified cholesterol was more frequently diminished both in concentration and in percentage of total cholesterol. Single determinations were of no value in distinguishing cirrhosis with transient superimposed "hepatitis" from terminal hepatic failure.

CONCLUSIONS AND SUMMARY

Application of five tests to the study of liver function in 228 patients under routine conditions in a large general hospital has demonstrated their value as a supplement to clinical methods in the diagnosis of cirrhosis and

other diseases of the liver. For the detection of liver disease before it had reached an advanced stage, the bromsulphalein retention test, the Takata Ara reaction, and fractionation of serum proteins, were more useful than icterus index and cholesterol or cholesterol ester determinations.

Discrimination should be exercised in the selection of tests, when doubt exists it is desirable to employ several tests in conjunction with one another.

The rate of excretion of bromsulphalein by normal individuals has been reexamined.

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ANALYSIS OF FLUID FROM A CHYLOUS MESENTERIC CYST*

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CHYLOUS cysts of the human mesentery are not of common occurrence, and chemical analyses of their contents are encountered in the literature only rarely. Since the cause of these cysts is still uncertain, it seemed desirable to report the following work:

REPORT OF CASE⁹

A forty-eight-year-old woman was admitted to the Surgical Service of St. Margaret Memorial Hospital, complaining of a gradual increase in the size of her abdomen, with accompanying discomfort, over a period of about four years. Palpation revealed a tumor in the left upper quadrant which was freely movable from side to side. It was painless, cystic in feel, and gave a flat note on percussion. Exploratory laparotomy disclosed a tumor mass 29 cm. in diameter. The cyst originated in the mesentery, close to its spinal attachment, at about the level of the first lumbar vertebra. Since a loop of small intestine was densely incorporated in the wall of the cyst, and the aorta and vena cava seemed to be intimately connected with its site of attachment, marsupialization was resorted to. About 1,000 c.c. of fluid,

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presently to be described, was aspirated from the cyst through a trocar and cannula. The trocar opening was enlarged, the cyst drawn out of the abdomen through the incision, and its walls sutured to the abdominal wall. The incision was then closed above and below the cyst, and the latter packed with gauze. The patient made a satisfactory recovery, the sinus formed by the cyst cavity eventually becoming completely obliterated.

On April 21, 1936, the sample of fluid aspirated at operation was submitted to the laboratory for analysis. It consisted of 750 cc of a thick, creamy fluid, out of which, on standing, settled a white, tenacious caseous sludge. In order to avoid interference with analytical procedures and to observe the purported resistance of chylous fluids to putrefaction, no preservative was added. The flask, which had hitherto been provided with a cotton plug, was stoppered with a clean cork (to prevent evaporation), and kept, when not in use, in an ice refrigerator.

ANALYTICAL METHODS

Before sampling, the contents of the flask were shaken vigorously for about two minutes, and the bottom of the flask examined for adherent sludge, to make certain that all solid matter was thoroughly and uniformly suspended. The sedimentation rate of the sludge was slow, consequently, a thorough agitation when the flask was removed from the refrigerator, and a briefer shaking prior to the pipetting of each sample, insured homogeneity of the samples.

Specific Gravity was determined by weighing, in a tared flask, a pipetted 5 cc sample of the fluid, and comparing its weight with that of 5 cc of water at the same temperature.

Total Solids—25 cc portions of the fluid were evaporated to a paste on a water bath in tared dishes, and dried to a weight constant within 1 mg under a vacuum of 60 to 120 mm of mercury.

Ash—The dried residues from the above determination were transferred, with the aid of a little water, to tared crucibles, and ignited to constant weight.

Total Nitrogen—5 cc of the fluid were diluted to 100 cc, and nitrogen determined on 1 cc aliquots of this solution, using Van Slyke's manometric hypobromite method.⁴

Nonprotein Nitrogen—5 cc samples of cyst fluid were precipitated by Haden's modification of the tungstic acid method of Fohn and Wu, and the nitrogen in aliquots of 1 to 5 cc of the filtrate, determined by the method used for total nitrogen.

Protein Nitrogen was calculated as the difference between total and nonprotein nitrogen.

Protein was calculated from protein nitrogen, using the customary factor of 6.25.

Lipids—Total fat, free fatty acids, soaps, and neutral fat, were determined on 5 cc portions of the fluid, using Fowweather's modification of Saxon's method for lipids in stools.⁶ Because excessive emulsification occurred when the fluid was extracted by shaking with alcohol and ether, a microconstant extraction apparatus was adapted for this purpose.

Calcium—5 cc of cyst fluid were digested with a sulphuric nitric acid mixture until clear, and made up to 100 cc. Aliquots of this mixture, neutralized to phenol red with dilute ammonia, were analyzed for calcium by the micromethod of Tisdall and Cramer, modified by Stanford and Wheatley.⁷

RESULTS

Results of the present analysis are listed in Table I, which includes for comparison, analytical figures for chylous cysts, chylous effusion, and chyle, taken from the literature.

DISCUSSION

Analytical procedures have not been specialized for chyle as for blood and urine. It was consequently necessary to adapt standard clinical laboratory methods to the purpose of this examination. Although these may not be thought to yield with chylous fluids a degree of accuracy as high as with

TABLE I
ANALYSES OF CHYLOUS FLUIDS

	CHYLOUS CYSTS					CHYLOUS DIFFUSION		CHYLE	
	AUTHOR'S CASE	SCHUMMS	ZDAREK ¹²	MORIOT AND JENNESSEAU ²		BUCHTALA ¹	PANZER ³		SOLIMAN ¹⁰
Volume c.c.	750 (1000)	1500	70	700		-	-	-	-
Specific Gravity	1.0120	0.988	-	1.004		1.017	-	-	-
			<i>Per Cent</i>						
Total Solids	12.45	39.8	10.58	13.18		8.66	9.71		7.10
Ash	1.85	0.92	0.84	0.95		0.95	0.81		0.60
Calcium	0.486	0.15	-	-		0.09	-		-
Total Nitrogen	0.786	-	-	-		0.750	-		-
Nonprotein Nitrogen	1.60	-	-	-		-	-		-
Protein Nitrogen	0.608	-	-	-		-	-		-
Total Fat	3.84	1.97	7.19	8.51		4.69	2.16		1.85
Free Fatty Acids	4.50	35.8	2.70	4.66		1.147	6.59		1.93
Soaps	1.30	-	-	-		-	-		-
Neutral Fat	1.50	0.43	-	-		-	-		-
	1.70	-	-	-		-	-		-

the materials for which they were intended, it is nevertheless felt that they are sufficiently exact to permit comparison between the composition of the chylous fluid here described, and the results found by other workers

Since several days had elapsed between the collection of the fluid and the beginning of the analysis, determination of sugars was not attempted. It is highly probable that any sugar originally present would have been more or less completely destroyed by glycolysis.

As may be seen in Table I, there is a wide range of variation in the figures reported by the various workers who have analyzed chylous fluids. This may be due partly to differences in analytical methods, but can be satisfactorily explained by the innate variability in composition of chylous fluids, and the effect of various factors of dehydration, differential absorption, etc., acting on a stagnant fluid. With a few exceptions, noted presently, the present figures agree well with those previously reported.

In determining protein we have assumed that the nitrogen precipitated by tungstic acid under the conditions of the customary Folm Wu blood precipitation method and protein nitrogen were identical. Solhman¹⁰ and Buchtala¹ calculated protein from total nitrogen alone, while the other workers cited here weighed coagulable matter and reported it as protein. Each of these methods is open to error when dealing with proteins of unknown properties. The relatively large amount of nonprotein nitrogen found suggests that a considerable amount of proteinlike substance escaped precipitation.

The author's figure for lipids lies well within the range of values previously reported. Although we lack sufficient data for comparison, the amount of soaps seems high. Because of the high calcium content of the fluid, one assumes the soap fraction to consist mainly of calcium soaps.

The feature which distinguishes the composition of the fluid under discussion from that of the other listed fluids is its high mineral content, and the high proportion of the latter represented by calcium. Of the 486 mg per cent of calcium found, only 109 mg would be necessary to combine with the fatty acids of the soap fraction, assuming the latter to be calcium palmitate. It seems possible that the high mineral content may, perhaps, indicate a beginning calcification of the contents of the cyst.

The ash, protein, and total lipids found comprise 82 per cent of the total solids, while, if the nitrogenous bodies not precipitated by tungstic acid (non-protein nitrogen fraction) were assumed to be proteinlike, 90 per cent of the solids would be accounted for.

BACTERIOSTATIC ACTION OF THE FLUID

Wells¹¹ points out that chylous effusions possess marked bacteriostatic properties, and the phenomenon is also mentioned by Morlot and Jennesseaux.¹² The author's experience corroborates these observations. Although no further effort was made to keep the fluid studied in this work sterile than in general, to store it in a refrigerator when not in use, it remained for over two months without showing gross evidence of putrefaction or fermentation. Cultures taken from it on May 22 showed a few colonies of gram negative bacilli (saprophytes?). On June 25 a faint odor of putrefaction was first noticed.

That a fluid of relatively high protein content could remain so long unputrefied, in spite of ample opportunity for contamination, and in the presence of a proved bacterial contamination, cannot be explained by the refrigeration to which it had been subjected. The temperature in the refrigerator averaged 10° C, and there were occasions when the flask containing the fluid was, by oversight, allowed to remain from eighteen to twenty-four hours in a room whose temperature varied from 25° to 30° C. It seems evident that the chylous fluid we studied possessed bacteriostatic activity which, toward the end of a two months' period, gradually disappeared; either by chemical change, or by being overwhelmed by successive bacterial contaminations.

SUMMARY AND CONCLUSIONS

1. The results of a chemical analysis of fluid from a chylous mesenteric cyst are reported.

2. With the exception of high values for ash and calcium, these results agree with previously reported analyses of chylous cyst contents and other chylous fluids.

3. It is suggested that the high content of inorganic salts, including calcium, may possibly be due to beginning calcification of the cyst.

4. The observation of others, that chylous fluids possess bacteriostatic activity, is confirmed for the fluid studied in this work.

The author wishes to acknowledge the cooperation of Dr. M. A. Sloeum, of the Surgical Department of St. Margaret Memorial Hospital, for placing the fluid at his disposal for analysis, and for furnishing the surgical and clinical data relating to the patient from whom it was obtained.

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THE EFFECTIVENESS OF THERAPEUTIC MEASURES AFTER POISONING WITH SUBLETHAL AND LETHAL DOSAGES OF BARBITAL IN THE RABBIT*

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THE efficacy of analeptics in the presence of poisoning from hypnotics or narcotics bears an inverse relation to the duration of action and depths of hypnosis of the depressant agent, according to Maloney, Fitch, and Tatum (1931), Maloney and Tatum (1932), and Swanson (1932). Resuscitation measures are thus symptomatically more effective against the short acting hypnotics of the pentobarbital, amytal, or avertin type (Barlow, 1935), than against barbitol per se (Maloney and Tatum, 1932, Lanegai, Dille, and Koppanyi 1935). Gros and Hofmann (1936), on the other hand, have shown that combined therapy with optimal dosages of metrazol (pentamethylene tetrazol) and ephedrine reduces the mortality of an 80 per cent lethal dosage of barbitol sodium in rats to zero. The significantly lower efficiency of analeptics in general, under conditions of barbitol or phenobarbital poisoning, as compared with other hypnotics of the same series, is due largely to the greater stability of the long acting preparations. These compounds are firmly fixed in the tissues and are excreted largely unchanged over a period of seven to nine days.

The generally unfavorable prognosis under clinical conditions of poisoning with lethal dosages of barbitol under present methods of therapy suggested the need of further study of the problem.

METHODS

Adult albino rabbits, weighing 2.5 to 3.5 kg. were used in all experiments. The diet fed for at least three weeks before medication of any animal included alfalfa hay, oats, and water ad lib. Fresh clover and lettuce were added twice weekly.

The hypnotic was administered intraperitoneally as a 10 per cent aqueous solution of barbitol sodium (Merck and Company) in each dosage series tested.

The efficacy of each agent and of combinations of the more satisfactory antidotal measures was tested under conditions of minimally hypnotic dosages (150 mg. per kg.), maximally hypnotic and exceptionally lethal dosages (300 mg. per kg.), a usually lethal (85 per cent) dosage of 450 mg. per kg., and after medication with not less than two absolute lethal dosages (1,000 mg. per kg.) of barbitol sodium.

The therapeutic agents or measures tested included "coramine" (NN dimethyl meotinamide), ephedrine, metrazol (pentamethylene tetrazol), piero-

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toxin, caffeine sodio-benzoate, the intravenous administration of dextrose, and combinations of the more effective agents of this group (Barlow, 1935).

Treatment was instituted in each series of experiments at the time hypnosis became maximal or respiratory depression became marked, i.e., one hour after medication with minimal dosages, and within fifteen to twenty minutes after maximal dosages of the hypnotic.

Therapy during the acute stages of poisoning was invariably by the intravenous route. In later stages of treatment intramuscular routes of medication were used. All animals, with the exception of those in the lowest dosage group, were treated after medication with the hypnotic at intervals as required by their condition up to forty-eight hours. Exceptionally in the lethal dosage groups, additional treatment up to seventy-two hours was required.

RESULTS

The antidotal values of different dosages of the several analeptics against the depression of minimal hypnotic dosages (150 mg. per kg.) of barbital sodium were determined on groups of 7 to 15 rabbits each. The control reactions of individual animals of each group to barbital alone were tested at least twice at ten-day intervals before the antidotal effects of any analeptic were studied. The observations made during the course of recovery from hypnosis included the time of spontaneous lifting of the head, sitting, and complete recovery. The final observation is most dependent on the experience of the observer, in that reliable end points are only obtainable by careful observations of spontaneous activity, equilibrium, etc., when the animals are free to move about in the absence of noise or other disturbing factors. All data were reduced to medians. Only the reactions following treatment (over a period of two to eight hours) with optimal dosages of each medication sequence will be discussed.

Maximal hypnosis following the intraperitoneal injection of barbital sodium in a dosage of 150 mg. per kg. occurred within 0.77 to 1.15 hours. Individual animals were relatively standard in their reactions. The initial stage of recovery in untreated controls (spontaneous lifting of the head) occurred within a range of 2 to 3.25 hours with a median of 2.3 hours after medication.

The evaluation of the several therapeutic measures differs somewhat, depending upon the time at which the different stages of recovery occur. Thus the order of efficiency from greatest to least as to the "awakening" effects is as follows: metrazol > picrotoxin and ephedrine > picrotoxin > ephedrine > coramine > caffeine > dextrose infusion. On the basis of the degree of shortening of the normal sitting or hopping time, the observed order of efficiency was as follows: metrazol > picrotoxin and ephedrine > ephedrine > picrotoxin > coramine > caffeine > dextrose. On the basis of the degree of shortening of the normal time for complete recovery from the hypnotic, the observed order was: metrazol and ephedrine, picrotoxin and ephedrine, picrotoxin, metrazol, ephedrine, dextrose infusion, coramine, caffeine. The latter two agents had no apparent shortening action on the normal complete recovery time.

Optimal antagonistic dosages of the several analeptics (in milligrams per kilogram) administered in four to six divided doses after the development of maximal sedative reactions to barbital are as follows: metrazol (250), picrotoxin (5), ephedrine (15), caffeine (150 to 250), coramine (450 to 750), dextrose (75 to 100 c.c. per kg. of a 5 per cent solution by vein), picrotoxin (5), and ephedrine (10).

Maloney and Tatum (1932) have reported that a dosage of 300 mg. of barbital sodium intraperitoneally killed 83.4 per cent of a series of 6 rabbits. The lethal effects of such dosages were reduced to 33 per cent under treatment with picrotoxin. Results of the present study indicate, due presumably to racial variability, that a dosage of 300 mg. of barbital per kg. killed only 2 of a series of 11 albino rabbits. This lethal figure was reduced to zero in a total of 36 rabbits by careful therapy at intervals of groups of 6 to 8 animals, with optimal dosages of metrazol (400 to 500 mg. per kg.), metrazol and ephedrine (15 mg. per kg.), picrotoxin (15 mg. per kg.), or dextrose (75 to 100 c.c. per kg. of a 5 per cent solution) intravenously. On the other hand, medication with effective dosages of caffeine appeared to be actually detrimental, i.e., the mortality under such therapy exceeded that of the control series.

The usually accepted minimum lethal dosage, i.e., that which kills 50 per cent of a series, was considered quite unsatisfactory from the standpoint of making an accurate evaluation of antidotal measures, in that under conditions of treatment, a 100 per cent survival would represent only a 50 per cent efficiency for the therapeutic measures under test.

The difficulties involved as to the interpretation of results of various antidotal measures after intraperitoneal injections of a 50 per cent lethal dosage of barbital sodium are illustrated by the variability of this figure in the hands of different observers. Using mixed breeds of rabbits, Fitch and McCandless reported that a dosage of 250 mg. of barbital sodium intraperitoneally killed 60 per cent of a series. Maloney and Tatum (1932) observed that 300 mg. per kg. killed 83.4 per cent. Jones and Lynn (1935) reported that 375 mg. per kg. killed only 50 per cent of a small series of animals, but a dosage of 385 mg. per kg. was 100 per cent lethal.

It was deemed necessary, therefore, to determine the toxicity of barbital sodium on intraperitoneal administration as a basis for a proper evaluation of the analeptic measures under study. The dosages tested ranged from 0.25 to 1.0 gm. per kg. body weight. All dosage groups included a total of 70 animals. None of the groups had received any of the barbituric acid derivatives previous to this test. These data indicate that 0.25 gm. per kg. killed only one of ten animals, 0.3 gm. per kg. killed 2 of 11 animals, and 0.4 gm. killed 4 of 11 animals. The 60 per cent MLD is 0.425 gm. per kg. The absolute lethal dosage by this route exceeds 0.45 gm. per kg. (killed 90 per cent of a series of 20 animals).

150 mg. per kg.—The first group of animals in the treated series of experiments was medicated with metrazol over a period of only twelve hours. All animals were nearly normal twelve hours after the beginning of treatment. Due to lack of further treatment, relapses occurred and all animals were dead on return to the laboratory at the twenty-second hour after barbital medication.

TABLE I
BARBITAL SODIUM (10% SOLUTION) 450 Mg./Kg. INTRAPERITONEALLY

RABBIT'S PER SERIES	TREATMENT	TOTAL DOSAGE MG. OR C.C./KG.	TREATMENT TIME—HOURS	MEDIAN RECOVERY TIME—HOURS	RECOVERY PER CENT OF SERIES	TIME OF DEATH HOURS	DEATHS PER CENT OF SERIES	REMARKS
15	Metrazol	300	48	60	93.34	36	6.66	Single death was due to over-treatment with picrotoxin.
	Picrotoxin	10						
	Ephedrine	7½						
11	Glucose 5%	75 c.c.						
	Picrotoxin	15						
11	Metrazol	500	48	72	90.81	47	9.09	
	Picrotoxin	36						
17	Glucose 10%	75-100 c.c.	48-60	72	90.81	39	9.09	
	Metrazol	1000-1400	28	72	83.3	64.5	16.6	
10	Metrazol	600-800	48-50	72-84	80	56	20	
	Glucose 5%	100 c.c.	30			(39-59½)		
11	Picrotoxin	16-20	60	72	71.8	40	28.2	
	Glucose 5%*					(36-43)		
16	Glucose 5%*	100 c.c.	36	72	50	15	50	
5	Coramine	750	During survival	--	0	(8-46)	100	Significant secondary depression.
	Controls	---		65.85 (60-120)	10	13½ (5½-26)		
20	Controls	---	----			11½ (3½-12)	90	

*Total volume of sugar solution was injected by vein in 4 to 6 divided doses.

The efficacy of various therapeutic measures, following the intraperitoneal injection of the 90 per cent lethal dosage of barbital sodium is illustrated in Table I. It was possible by careful symptomatic control of respiration and indirect control of the circulation, by means of a combined therapy with picrotoxin, metrazol, ephedrine, and 5 per cent glucose by vein, to save 93.3 per cent of a series of 15 animals, while medication with picrotoxin and metrazol, or picrotoxin and 5 per cent glucose by vein, saved similar percentages (90.8) of groups of 11 animals.

The intravenous administration of 5 per cent dextrose at intervals over a period of forty hours was of some antidotal value, in that under such treatment, more than twice as many survivals occurred as were noted in the control series. The effectiveness of fluids was further demonstrated when this procedure was combined with coramine. Such combined therapy saved 50 per cent of the treated animals, although all animals of a second group treated with coramine alone died. Effective dosages of coramine were prone to produce a significant secondary depression, generally accompanied by pulmonary edema.

The mechanism of action of the intravenous glucose in decreasing the lethal effects of barbital is explained only in part. Unpublished data on dogs indicate that 10 per cent glucose by vein results in a small but significant acceleration of the normal urinary excretion rate of barbital. The diuretic effects of the injected fluid on rabbits were marked. Koppányi, Murphy, and Kiop (1933) observed no acceleration of the barbital excretion rate in dogs when treated with 10 per cent glucose by vein. Johnson, Luckhardt, and Lighthill (1930) reported that the duration of barbital hypnosis in dogs was reduced 50 per cent by intravenous glucose medication. The period required for complete recovery of barbitalized rabbits under the fluid therapy was not significantly altered, although the depth of hypnosis was significantly diminished. These data suggest that 5 per cent dextrose is superior to higher concentrations in the volume injected. The primary beneficial effects of isotonic glucose by vein are visualized as maintaining a positive water balance and the prevention of the development of acidosis as reported by Henze (1936), and Fischer and Salzer (1936).

1,000 mg per kg—The median time of death in a series of 7 rabbits medicated intraperitoneally with a dosage of one gram of barbital sodium per kilogram body weight, considered to be twice the absolute lethal dosage for rabbits, was thirty-eight minutes, with a range of twenty-five to sixty-nine minutes.

The order of efficiency from greatest to least of the most satisfactory analeptic measures tested based on data obtained from the treatment of poisonings with smaller dosages of barbital, is illustrated in Table II.

All types of symptomatic treatment were effective in prolonging the survival period, in that death occurred only after eleven hours in the series treated with the least effective therapeutic sequence, as compared with a median of thirty-eight minutes in the control or untreated group. In other words control of respiration and indirectly of the circulation with picrotoxin, metrazol, and ephedrine, together with fluids (5 per cent glucose) intravenously as required over a period of thirty-six to sixty hours, reduced the

TABLE II
BARBITAL SODIUM 1.0 GM./Kg. INTRAPERITONEALLY

NUMBER OF RABBITS PER GROUP	SERIES	TREATMENT	TOTAL DOSAGE MG. OR C.C./KG.	NUMBER OF DOSES	TREAT- MENT TIME HOURS	RECOVERY PER CENT OF SERIES	TIME OF COMPLETE RECOVERY DAYS	DEATHS PER CENT OF SERIES	TIME OF DEATH HOURS		REMARKS
									MEDIAN	RANGE	
7	1	Controls	--	--	--	0.0	--	100.0	0.63	0.4-1.15	
6	2	Picrotoxin	39	11-16	48	83.3	4.5	16.7	39.5		
		Metrazol	1800-2200	16-22	48						
		Ephedrine Glucose 5%	20 90-125 c.c.	4 4-6	36 36						
11	3	Picrotoxin Glucose 5%	21 130 c.c.	10-16 7	50	63.63	31.5	36.37	21	19-38	One animal died due to insufficient treatment
11	4	Picrotoxin	25-37	18	48-60	54.55	5	45.45	20	12.2-20	
11	5	Picrotoxin Metrazol Ephedrine Glucose 5%	36 1,450 25 100 c.c.	16 18 4 4	48 60 36 36	30	41	70	16.5	11-38.5	No treatment be- tween 12th and 23rd hours; 5 out of 7 died during this time
11	6	Same as series 5	No treatment between 5th and 12th hours					81.82	7 out of 9 died between the 6th and 12th hours.		

mortality from 100 per cent to 16.7 per cent. The method of treatment used in the initial experiments of this dosage group was a duplication of the above therapeutic sequence, with the exception that no treatment was given between the fifth and twelfth hours due to the apparently excellent condition of the animals. The survival percentage of this initial series was only 18.8 per cent, i.e., seven of the nine fatalities occurred during this period because of insufficient medication. This picture is almost duplicated by discontinuance of the therapy between the twelfth and twenty-third hours after the injection of the hypnotic.

DISCUSSION

Symptomatic control of the effects of barbital sodium poisoning in rabbits appears to be best accomplished by means of frequent medication with two central convulsants—picrotoxin and metiazol, the judicious administration of a vasopressor agent, ephedrine, and the administration of fluids intravenously preferably in the form of 5 per cent glucose. The efficacy of fluid in a corresponding volume of 10 per cent glucose or Ringer's solution was inferior to isotonic glucose. Medication should be continued at short intervals at the outset of the poisoning as judged by the symptoms present, and at longer intervals as the depth of hypnosis diminishes. In the presence of one or more absolute lethal dosages treatment should be continued for a period of at least thirty-six hours for optimal results. The intervals of treatment depend on the condition of individual animals.

The administration of picrotoxin results in a submaximal stimulation of respiration, and reactions become maximal only after a latent period. The product in these respects is inferior to metiazol for emergency purposes. However, the duration of action of picrotoxin which ranges from thirty to one hundred eighty minutes depending on the depth of hypnosis, is superior to that of metiazol. On the other hand, picrotoxin is not wholly free of undesirable side actions, in that significant secondary depressant effects may develop after excessive dosages.

The respiratory stimulant effects of metiazol were definitely superior to those of other agents tested. Its duration of action may be as short as ten minutes in poisoning with massive dosages of pentobarbital, chloral, avertin, or barbital but generally persists for twenty-five to forty-five minutes in lighter grades of poisoning. This product is best suited for emergency purposes under conditions of respiratory failure. On numerous occasions spontaneous respiratory movements have been initiated with metiazol intravenously in the presence of continued heart action, one or two minutes after cessation of respiration. An optimal sequence of agents for use in emergencies of respiratory depression or arrest would be a combination of both metiazol and picrotoxin. This sequence would result in a prompt and marked stimulation (ten to thirty minutes) of respiration by metiazol which in turn would be summated slightly and prolonged by the additional medication with picrotoxin. The optimal therapeutic level is best judged by maintenance of a good color (absence of anoxemia) and an abnormal reflex excitability. The effects of excessive dosages of either of these two analeptics are readily controlled by pentobarbital by vein.

Ephedrine injected intravenously in maximal dosages of 2.5 to 5.0 mg. per kg. has a definite place in the antidotal sequence against hypnotic or narcotic poisoning. The optimal treatment interval with such dosages in rabbits appears to be between three and six hours. Larger dosages of ephedrine are prone to produce a heart block of greater or less degree and an embarrassment of respiration due to the development of pulmonary edema.

The administration of fluids by vein relieves the anuric effects of barbital, accelerates the urinary excretion of barbital slightly, maintains a more normal water balance (in that animals poisoned with barbital do not eat and very rarely drink water during the thirty to fifty hours after poisoning), and very probably reduces the tendency for the development of acidosis (Henze, 1936; Fischer and Salzer, 1936; Chang and Tainter, 1936; and Sack, 1936). Recovery is invariably characterized by the ingestion of water freely. Fluids alone are of definite symptomatic value in that the depth of lighter grades of hypnosis is diminished. The time required for complete recovery after such therapy alone appears to be little altered as observed by Riedel, 1930, although the mortality figures are significantly reduced. Optimal results are obtained when fluids are administered in conjunction with picrotoxin, metrazol, and ephedrine.

"Coramine," strychnine, and caffeine were of questionable value in the treatment of even lighter grades of barbital sodium poisoning. The order of observed efficiency corresponds to that in which the compounds are listed, as a matter of fact, the data obtained indicate undesirable effects, such as secondary depression from effective dosages, and, in the case of caffeine, these toxic effects were additive with those of the hypnotic.

CONCLUSIONS

The relative antidotal values of a series of analeptic measures have been tested on the rabbit after intraperitoneal medication with maximally sedative, maximally hypnotic, single lethal, and at least two lethal dosages of barbital sodium.

Control of the respiratory symptoms of poisoning by medication at suitable intervals for a period of thirty-six to forty-eight hours, with picrotoxin and metrazol, and of the circulation by means of ephedrine, together with fluids intravenously, prolonged the median period of survival after two lethal dosages of barbital from thirty-eight minutes (control) to a minimum of 39.5 hours and reduced the mortality from 100 per cent to 16.7 per cent.

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BACTERIOSTATIC ACTION OF PRONTOSIL SOLUBLE, SULFANILAMIDE, AND DISULFANILAMIDE ON THE SPORULATING ANAEROBES COMMONLY CAUSALLY ASSOCIATED WITH GASEOUS GANGRENE*

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IT IS impractical and unessential here to review the already voluminous literature on the nature and action of the sulfanilamide series in the treatment of various infections. This action has already been rather conclusively demonstrated experimentally against the streptococcus and pneumococcus (at least certain types), the gonococcus, the meningococcus and more recently against malaria and the filterable virus of lymphocytic choriomeningitis.

As far as we can ascertain, however, only one study records the clinical action against the sporulating anaerobes of human gaseous gangrene. Bohlman¹ reports the treatment with sulfanilamide of three cases with "an amazing not to say dramatic, result in a desperate case of gas gangrene. Cases 2 and 3 are confirmatory." *Clostridium welchii* was actually isolated only from Case 3, but was presumably present in all cases, either from symptoms or microscopic observation.

Although it is customary to precede clinical trial by some form of experimental *in vitro* test of a drug, no record of such test on the sporulating anaerobes

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of gaseous gangrene has yet been observed in the current literature. Bohlman does quote a personal communication from P. H. Long, who is stated to be "conducting experiments with *B. welchii* to determine the effect of Sulfanilamide on growth and phagocytosis."

In view of this lack of proof of direct, or primary bacteriostatic action of sulfanilamide on the sporulating anaerobes, it appears desirable to attempt such measurements of sulfanilamide and related compounds. To this end we obtained, through the courtesy of the Winthrop Chemical Company, Inc., experimental lots of prontosil soluble powder and of prontosil soluble (2.5 per cent solution). From Parke, Davis Company Research Laboratories we were provided with sulfanilamide and disulfanilamide.²

As test organisms we selected, from an extensive collection of sporulating anaerobes, a series of species commonly encountered, either alone or in combination, in human gaseous gangrene. These selected species were: *Cl. welchii*, *Cl. tertium*, *Cl. sporogenes*, *Cl. tetani*, *Cl. lentoputrescens*, *Cl. bifermentans*, *Cl. novyi*, *Cl. septicum*, and *Cl. histolyticum*. In addition was included *Cl. botulinum* Type A, although this organism is not associated with gaseous gangrene. Stock cultures of these organisms were grown in semisolid agar for twenty-four hours just preceding the tests.

Preliminary tests were conducted in a simple medium composed of 1 per cent Baeto-Tryptone, 1 per cent Baeto-Neopeptone and 0.2 per cent dextrose in distilled water. To this basic medium the several drugs were added in serial dilutions, which were then tubed in 7 by $\frac{1}{2}$ inch tubes, each covered with a heavy oil seal 1 cm. deep, and autoclaved at 120° C. for twenty minutes. They were cooled quickly and inoculated immediately. The reaction of the medium, as inoculated, was about pH 6.8 to 6.9, and was not visibly altered by the drugs in the dilutions tested.

Each test dilution was inoculated, through the oil seal, with a Pasteur pipette from the twenty-four-hour-old stock culture in semisolid agar. Approximately 0.05 c.c. was delivered deep into each tube. All cultures were incubated at 37° C., and were observed at suitable intervals. Readings were recorded in terms of perceptible turbidity as compared with the control inoculation into the basic medium without added drug. The turbidity of the control culture is in all cases considered "4 plus," although the turbidity of *Cl. tetani*, for example, was much less than that of *Cl. sporogenes* or *Cl. bifermentans*. Inhibition of growth must, therefore, be judged by comparing each drug dilution culture with its own control.

The results of several repetitions are summarized in Table I. We should observe here that, while absolutely equivalent turbidities were not obtained, nor expected, on repetitions of tests of the same dilutions, there was a surprisingly high correlation.

From Table I it appears that prontosil soluble (2.5 per cent solution) exerts a direct bacteriostatic action, to and including a dilution of 1:1000, upon

We desire to express appreciation to the Winthrop Chemical Company, Inc., and to Parke, Davis Company for the cooperation which made possible this study.

Cl tetani, *Cl lentoputrescens*, *Cl novyi*, *Cl septicum* and *Cl histolyticum* The other species tested were scarcely, if at all, affected until exposed to a concentration of 1 125

It might be of interest to note that all species reduced the red color of the ding to a colorless state whenever growth occurred to a degree of marked turbidity Some species were more active in this respect than others Thus, in one test, *Cl welchii* completely decolorized the 1 250 dilution, while *Cl sporogenes* and others decolorized the 1 2000 but not the 1 1000 dilution

Sulfanilamide exerted almost an identical degree of inhibition as did the prontosil soluble Slight differences in turbidity are recorded, but these vary almost as much for one as for the other drug Little, if any, more effective inhibition was observable with sulfanilamide, although such might have been expected from the chemical structure

TABLE I

PRELIMINARY TEST OF SULFANILAMIDE DISULFANILAMIDE AND PRONTOSIL SOLUBLE IN DECAIOSE PEPTONE WATER RECORDED AFTER 66 HOURS' INCUBATION

CULTURES TESTED	SULFANILAMIDE DILUTIONS						DISULFANILAMIDE DILUTIONS						PRONTOSIL SOLUBLE DILUTIONS						CONTROL NO BUG				
	1 125	1 250	1 500	1 1000	1 2000	1 4000	1 8000	1 125	1 250	1 500	1 1000	1 2000	1 4000	1 8000	1 125	1 250	1 500	1 1000		1 2000	1 4000	1 8000	
<i>Cl welchii</i> *	3	4	4	4	4	4	4	0	0	0	1	4	4	4	1	3	4	4	4	4	4	4	+
<i>Cl tertium</i>	0	3	3	4	4	4	4	0	0	1	2	4	4	4	2	3	4	4	4	4	4	4	+
<i>Cl sporogenes</i>	4	4	4	4	4	4	4	0	0	0	4	4	4	4	1	3	4	4	4	4	4	4	+
<i>Cl tetani</i>	0	0	0	1	2	3	4	0	0	0	0	1	2	4	0	0	0	1	3	4	4	4	+
<i>Cl lentoputrescens</i>	0	0	0	0	2	4	4	0	0	0	0	1	1	2	0	0	0	0	3	4	4	4	+
<i>Cl bifermentans</i>	2	4	4	4	4	4	4	0	0	2	4	4	4	4	2	3	4	4	4	4	4	4	+
<i>Cl novyi</i>	0	0	0	0	2	3	4	0	0	0	0	0	0	4	0	0	0	2	4	4	4	4	+
<i>Cl septicum</i>	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	+
<i>Cl histolyticum</i>	0	0	0	0	1	3	4	0	0	0	0	1	4	4	0	0	0	1	4	4	4	4	+
<i>Cl batulum</i> Type A	2	3	4	4	4	4	4	0	0	1	4	4	4	4	1	2	3	3	4	4	4	4	+

*Numerals indicate degree of turbidity compared with that of the control

Disulfanilamide, on the contrary, effected a distinctly superior inhibition, completely suppressing all species to and including a dilution of 1 250 Again, also, a specific action was apparent on the same organisms as were affected by prontosil soluble and sulfanilamide Furthermore, this action was evident to higher dilutions, for example, *Cl septicum* was completely inhibited by a 1 8000 dilution

This test was then repeated, preparing dilutions from the prontosil powder The results were essentially identical with those obtained with dilutions of the prontosil soluble (2.5 per cent solution), except that similar numerical dilutions of the powder appeared very slightly less effective than those derived from the prontosil soluble solution This disparity, although slight, was uniformly and sufficiently evident to indicate a measurable and unexplained superiority of the action of the prontosil soluble (2.5 per cent solution)

In these above tests, it will be noted, the several drugs were added to the basic medium and sterilized by autoclaving Since this is contrary to the

therapeutic use, it is conceivable that their activity may be depressed by such sterilization. At the same time, in this type of experimentation, some form of sterilization is, of course, essential. Moreover, the exigencies of anaerobic cultivation demand at least the boiling of the culture medium before inoculation, in order to insure conditions proper for growth. In this respect, then, the results of these experiments cannot be directly compared with those which may be obtained by administering the same drugs, unaffected by heating, either by mouth or by injection into the human body.

To determine if "self-sterilization" might be obtained, dilutions of 1:250, 1:500, and 1:1000 were prepared in the peptone broth, tubed under oil, and incubated without sterilization. After twenty-four hours' incubation, all tubes were heavily turbid with various contaminants. Since 1:250 marks approximately the saturation of disulfanilamide in the basic medium, this method was obviously eliminated.

TABLE II

DISULFANILAMIDE AUTOCLAVED IN DISTILLED WATER AND ASEPTICALLY ADDED TO THE BASIC MEDIUM RECORDED AFTER 66 HOURS' INCUBATION

CULTURES TESTED	DILUTIONS				
	1:1000	1:2000	1:4000	1:8000	CONTROL
<i>Cl. welchii</i>	3	4	4	4	4
<i>Cl. tertium</i>	0	2	4	4	4
<i>Cl. sporogenes</i>	3	4	4	4	4
<i>Cl. tetani</i>	0	2	3	4	4
<i>Cl. lentoputrescens</i>	0	0	3	3	4
<i>Cl. bifermentans</i>	2	4	4	4	4
<i>Cl. novyi</i>	0	0	0	0	4
<i>Cl. septicum</i>	0	0	0	0	4
<i>Cl. histolyticum</i>	0	0	0	0	4
<i>Cl. botulinum</i> Type A	3	4	4	4	4

Filtration of the initial dilutions might be attempted. However, unless boiled and protected against the reabsorption of oxygen, the addition of these dilutions would reaccrute the test mediums. Due to these, and other technical complications, filtration experiments were not attempted.

We did, however, utilize a principle commonly applied to the sterilization of certain carbohydrates. The disulfanilamide was autoclaved in a 1:125 dilution in neutral distilled water. The basic peptone broth was tubed under oil in 9 c.c. amounts and autoclaved separately. This dilution of disulfanilamide is really a supersaturated solution, but remains in solution while hot. While still in solution, serial dilutions were made with sterile pipettes through 9 c.c. sterile distilled, and freshly boiled, distilled water blanks. These dilutions were then aseptically delivered, in 1 c.c. amounts, under the oil seal into the basic medium to effect the desired end dilutions. These tubes were then inoculated and incubated. The results are shown in Table II.

In comparing the results expressed in Table II with those in Table I it appears, superficially, that the autoclaving of disulfanilamide in distilled water

permits it to retain a slightly greater bacteriostatic activity. However, again the factor of necessary introduction of oxygen with the aerated dilutions of the drug might be sufficient to account for this.

It is a simple matter, using methylene blue as an indicator, to show that boiled distilled water becomes fully aerated within the time required to make the necessary dilutions and to deliver these into the basic medium. It would be difficult to evaluate this possible cause but at least no appreciable gain is observed by autoclaving the drug in distilled water, with subsequent addition to the medium.

Obviously these *in vitro* tests in a simple peptone medium do not approach the complexity of conditions within the human body. Nor is there any practicable way to attain them under conditions suitable for anaerobic cultivation.

TABLE III

TEST OF SULFANILAMIDE, DISULFANILAMIDE, AND PRONTOSIL SOLUBLE (25 PER CENT SOLUTION) IN DILUTE LOEFFLER'S SERUM DEXTROSE PEPTONE BROTH RECORDED AFTER 60 HOURS' INCUBATION

CULTURES TESTED	SULFANILAMIDE DILUTIONS					DISULFANILAMIDE DILUTIONS							PRONTOSIL SOLUBLE DILUTIONS					CONTROL NO DRUG			
	1:250	1:500	1:1000	1:2000	1:4000	1:250	1:500	1:1000	1:2000	1:4000	1:8000	1:16,000	1:32,000	1:250	1:500	1:1000	1:2000		1:4000	1:8000	
<i>Cl. welchii</i>	1	2	4	4	4	4	0	0	1	2	4	4	4	4	1	2	4	4	4	4	4
<i>Cl. tertium</i>	0	0	1	2	4	4	0	0	0	0	1	2	4	4	0	1	2	4	4	4	4
<i>Cl. sporogenes</i>	1	4	4	4	4	4	0	3	4	4	4	4	4	4	3	4	4	4	4	4	4
<i>Cl. tetani</i>	1	2	3	3	4	4	0	0	0	0	1	2	3	4	0	1	2	4	4	4	4
<i>Cl. lentoputrescens</i>	0	1	2	2	4	4	0	0	0	0	0	1	2	2	0	1	2	3	3	4	4
<i>Cl. bisfermentans</i>	1	2	4	4	4	4	0	0	0	1	2	4	4	4	1	2	4	4	4	4	4
<i>Cl. novyi</i>	0	0	0	1	3	5	0	0	0	0	0	1	2	4	0	1	2	3	3	4	4
<i>Cl. septicum</i>	0	0	1	2	4	4	0	0	0	0	1	2	4	4	0	0	1	2	4	4	4
<i>Cl. histolyticum</i>	1	2	3	4	4	4	0	0	1	2	3	4	4	4	0	2	4	4	4	4	4
<i>Cl. botulinum</i> Type A	3	4	4	4	4	4	0	1	3	4	4	4	4	4	2	4	4	4	4	4	4

We did simulate them, to a degree, by preparing a second basic medium composed of Difco Loeffler's blood serum dehydrated. One gram of this powder was dissolved in 140 c.c. of the stock dextrose peptone broth. In this medium the several drugs were diluted as before, tubed under oil, and autoclaved. The tubes contained, after autoclaving, a fibrinlike clot in a clear fluid.

Since disulfanilamide, in the previous tests, completely inhibited certain species to the maximum 1:8000 dilution, in this test it was diluted further to 1:32,000.

The tubes were inoculated, incubated, and observed for turbidity and decolorization of the prontosil soluble. The results are recorded in Table III.

When Table III is compared with Table I, it is evident that the action of the several drugs in dilute Loeffler's serum water is practically identical with that in the simple dextrose peptone broth. If anything, the activity in the serum water is slightly less, as noted particularly with the disulfanilamide. At least there is no distinct increase, such as is stated to occur in tests of acriflavine and certain similar compounds.

Finally, a series of tests was run in a semisolid agar prepared by adding 0.25 per cent agar to the dextrose-peptone water base. The several drugs were diluted as before and tubed without oil seal, autoclaved, inoculated, and incubated.

The readings in this semisolid agar so nearly exactly parallel those in the previous tests that it is not essential to present them in tabular form.

SUMMARY

We have attempted to measure, under several experimental conditions, the direct bacteriostatic action of sulfanilamide, disulfanilamide and prontosil soluble upon a series of sporulating anaerobes commonly causally associated, alone or in combination, with human gaseous gangrene.

These tests indicate that, under these test conditions, the drugs exert an apparently specific selective action against certain species, notably *Cl. tetani*, *Cl. lentoputrescens*, *Cl. novyi*, *Cl. septicum*, and *Cl. histolyticum*. On the contrary, *Cl. welchii* and the intensely proteolytic types such as *Cl. sporogenes* and *Cl. bifermentans*, as well as the toxic *Cl. botulinum* Type A, are scarcely at all affected, at least by any concentrations approaching those tolerated in therapeutic dosages of human beings.

It appears, from these tests, that the bacteriostatic activity increases in order: prontosil soluble, sulfanilamide, and disulfanilamide. This is in keeping with their chemical composition, and might be anticipated. In fact these results parallel those of Rosenthal, Bauer and Branham² in testing these same compounds for protection of mice against streptococcus and meningococcus infection.

Lastly, admitting Bohlman's¹ "dramatic" results in the treatment of 3 cases of presumably *Cl. welchii* infections with sulfanilamide, and comparing the experimental concentrations tested with the maximum estimated body concentrations attained by his dosages, it would seem necessary to assume some secondary therapeutic activity of the drug other than a simple and direct bacteriostatic action upon the bacteria themselves.

These experiments measure only the direct bacteriostatic activity, and we attempt here no explanation of the obvious discrepancy between the experimental results and the therapeutic records.

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THE URINARY EXCRETION OF SINGLE DOSES OF SULFANILAMIDE*

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THE successful treatment of urinary infections¹ with sulfanilamide has directed attention to the measurement of urinary concentrations of the drug,² since it has been pointed out that the therapeutic efficacy is a function of the concentration of the drug in urine. With a view to further elucidation of the elimination of the drug, we have administered single doses to three male subjects. F, a normal white male, weighing 125 pounds, was given 2.0 gm., L. T., a normal colored male, weighing 150 pounds, was given 2.5 gm., and A. F., a normal white male, weighing 125 pounds, was given 3.0 gm. A. F. became dizzy and nauseous three hours after ingestion of the drug. He forced fluids and was given 10 gram of sodium bicarbonate. These subjects were occupied with their normal routines. Urine samples were voided ad libitum, and analyzed for free and total sulfanilamide.

The data obtained are abbreviated in Table I to show only the maxima observed, and the number of hours after ingestion of the drug at which these occurred. Concentrations are expressed in milligrams per cent, and rates in milligrams per hour.

The rate of excretion reached apices which did not correspond to the concentration peaks, although maximal rates of excretion of free and total sulfanilamide occurred at the same time in all cases. In two cases an additional later peak was observed in the rate of total sulfanilamide excretion.

The maxima observed for conjugated sulfanilamide concentrations occurred later than the peaks for the free sulfanilamide. Consequently, the concentration maxima of the total sulfanilamide correspond to the maxima of the free, or conjugated sulfanilamide, or both.

The multiple peaks observed rise sharply, and are undoubtedly the result of the factors, viz., water intake, urinary pH, physical activity, tissue desorption, tubular resorption,³ etc.

Excretion of the drug, in the order of the cases presented, was complete in fifty-fifty-eight, and fifty-four hours. The total recovery of sulfanilamide was 53, 51, and 58 per cent of the amount ingested, respectively. Over all values were checked by diazotizing and coupling the combined urine samples in each case to overcome the colorimetric reading errors at low concentrations. The data were obtained in the third case, using Marshall's method,⁷ and in all other cases using the chromotrope acid method.⁸ The total recovery is in good agreement with the data obtained by Fuller⁹ in animals. Approximately 45 per cent of the initial dose of sulfanilamide remains unaccounted for. This

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may be partially excreted in another form, since Fuller could find only small amounts in feces, and Marshall¹⁰ recovered less than 2 per cent in the intestinal tract of dogs, four hours after ingestion of the drug.

The recovery of free sulfanilamide was 24, 17, and 37 per cent. The last value appears to be high and may be due to the diuresis induced.

TABLE I
SULFANILAMIDE

CASE	FREE				CONJUGATE		TOTAL			
	CONCENTRATION MAXIMA MG. %	TIME ATTAINED (HOURS AFTER INGESTION)	RATE MAXIMA MG. PER HOUR	TIME ATTAINED	CONCENTRATION MAXIMA MG. %	TIME ATTAINED (HOURS AFTER INGESTION)	CONCENTRATION MAXIMA MG. %	TIME ATTAINED (HOURS AFTER INGESTION)	RATE MAXIMA MG. PER HOUR	TIME ATTAINED
F.	35.1	3	40.3	3	26.3	7	61.2	17	58.6	4
	36.1	19	48.8	19	35.0	19	43.6	26	96.0	17
					28.4	26			24.0	26
L. T.	32.4	11	26.3	6	34.4	11	66.8	17	42.0	6
			21.4	33	45.1	48	56.8	48	63.3	33
A. F.	68.9	4	123.0	8	21.4	6	81.6	6	131.0	8
	55.1	19			31.1	19	86.2	19	50.0	19
					27.7	28	46.8	28		

It was noted that approximately 80 per cent of the free sulfanilamide excreted (80.0, 87.5, 75.4 per cent) was recovered during the first twenty-four hours, whereas only 60 per cent of the total sulfanilamide (65.0, 57.5, 63.1 per cent) was excreted during this time. The difference is due to a lag in the excretion of acetylsulfanilamide and was anticipated from the recession of the concentration peaks observed. This may result from a time requirement in the detoxification mechanism.

TABLE II

CASE	URINE VOLUME IN 1ST 24 HOURS	FREE SULFANILAMIDE IN GRAMS IN 1ST 24 HOURS	% RECOVERED AS FREE SULFANILAMIDE IN 1ST 24 HOURS	TOTAL GRAMS IN 24 HOURS	% RECOVERED OF TOTAL IN 1ST 24 HOURS
A. K.	857	0.181	3.6	0.412	8.2
J.	995	0.445	11.4	0.791	19.8
T.	2020	0.370	14.8	0.725	29.0
H.	2250	0.980	19.6	1.495	29.9
F.	1745	0.385	19.2	0.688	34.4
A. F.	2430	0.836	27.9	1.100	36.6

Since colorimetric comparison is more accurate at higher urinary concentrations of sulfanilamide, the following three male patients, recovering from tibia-fibula fractures, were studied during the first twenty-four hours only. The amount of sulfanilamide administered in these cases was increased to 4 and 5 gm. Helmholz and Osterberg² report that after a single 5-gram dose of the drug, the urine will at some time contain as high as 60 mg. per cent free and 100 mg. per cent conjugated sulfanilamide. One patient, A. K., weighing 130 pounds, and given 5 gm. of the drug, did not nearly approximate

these values. H, weighing 180 pounds, given 5 gm, achieved a free sulfanilamide concentration of 75 mg per cent but the conjugated form did not exceed 50 mg per cent. J, weighing 168 pounds, and given only 4 gm, fell in this range. A fourth case, G, not reported here in detail, with obvious kidney involvement, and given only 5 gm achieved concentrations of 80 mg per cent free and 175 mg per cent conjugated sulfanilamide.

Care was exercised to avoid urine losses in all cases. The recovery within the first twenty four hours of the free and total sulfanilamide expressed in per cent of the drug administered (see Table II) varied widely. It was observed that the low recovery was concomitant with low urinary volume.

The retention of sulfanilamide in the first two cases (A, K and J) is apparently not related to impaired kidney function, since both subjects were regarded clinically normal. The high value of free sulfanilamide in Case A, F (27.9 per cent) may be due to diuresis. These findings are in agreement with the results reported by Marshall.⁶ The amount of the drug excreted during the first twenty four hours is obviously not solely related to the urinary volume.

SUMMARY

Multiple peaks have been observed in the concentration and the rate of output of free and conjugated sulfanilamide in the urine following single doses of the drug. The rate and concentration maxima do not appear to be related.

Fifty five per cent (± 5 per cent) of the ingested sulfanilamide has been recovered.

The percentage of the drug recovered within the first twenty four hours varied from 8 to 36 per cent. The two lowest values were, among other things, associated with a low urinary output and were apparently not related to impaired kidney function.

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LABORATORY METHODS

A MICROCONSTANT EXTRACTION APPARATUS FOR LIQUIDS*

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IT IS frequently necessary to extract a small amount of fluid with a non-miscible solvent under conditions where mechanical separation is unsatisfactory. A wide variety of devices has been described for the automatic constant extraction of liquids with lighter, nonmiscible liquids;^{1, 2} but none is suitable for the extraction of volumes under 10 c.c., and most achieve efficiency at the cost of simplicity. From the latter standpoint, the apparatus described by Kurzrok and Ratner³ is an exception, and has formed the basis of design for the microapparatus described here.

The use of such an apparatus for the extraction of small amounts of fluid quantitatively has a threefold advantage over the use of a separatory funnel: The extraction is not attended with the mechanical losses encountered with the funnel in all but the most skilled hands; the extract is left in a small volume of solvent; and, the process being automatic, it is less time-consuming. Although the following description is somewhat specific in respect to details, the apparatus (since it can be made by anyone with reasonable skill in glass-blowing) lends itself to wide variation, adapted to specific needs.

Referring to Fig. 1, it will be seen that the apparatus consists essentially of a boiling chamber, *A*; a condensing chamber, *B*, fitted with a condenser; and an extracting chamber, *C*. The boiling chamber is fitted with an outlet tube, *E*, leading to *B*, and a return tube, *F*, leading from *C*. The condensation chamber terminates below in a narrow tube, *H*, reaching to the bottom of *C*, and carrying a sealed concentric outlet tube, *G*, into which *F* is fused.

The boiling chamber, in the case illustrated, is a 125 ml. Erlenmeyer flask; and the extraction chamber, a test tube 6 inches by $\frac{3}{4}$ inch. The condensation chamber is of 25 mm., and the vertical tube *H*, of 5 mm. glass tubing. The joint between *B* and *H* is best made with an intermediate section of tubing of larger diameter than *H*, as illustrated, for the sake of sturdiness. A bulb is blown on the lower end of *H*, containing minute perforations in its undersurface. All stoppers are cork of good quality. Construction is easier, and the apparatus less fragile, if tube *E* is made discontinuous, and provided with a joint of rubber tubing (*D*).

In operation, 50 to 75 c.c. of solvent is placed in *A*, and the fluid to be extracted, in *C*. The latter is then filled nearly to the top with solvent. The apparatus is connected, the condenser water started, and the solvent in flask *A*

*From the John C. Oliver Memorial Research Laboratory, St. Margaret Memorial Hospital, Pittsburgh.

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boiled over an appropriate source of heat. Vapor from the solvent reaches *B* through *E*, is condensed, and collects in *H* until the height of its column overbalances the pressure exerted by fluid and supernatant solvent in *C*. Then the solvent flows out through the perforations in the bulb at the end of *H*, passes up through the fluid in a fine state of subdivision, extracting as it goes, and joins the supernatant solvent. When the latter has reached the level of the return tube *F*, it flows back into *A*, carrying with it extracted material from *C*.

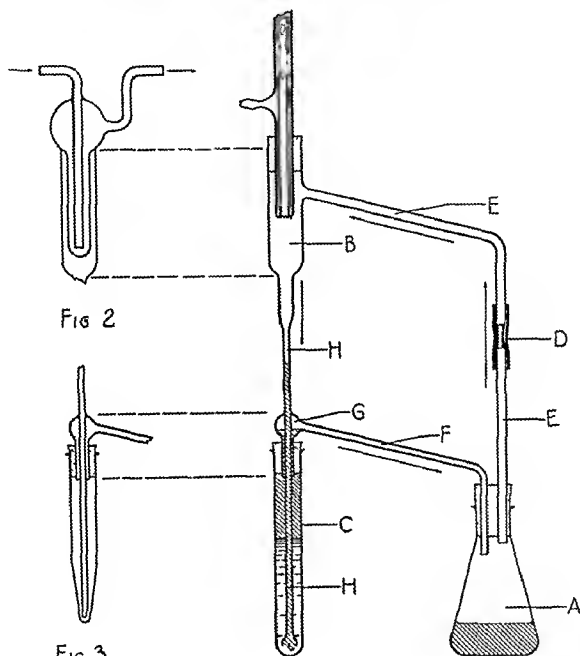


FIG 1

Figs 1 • 3

The fluid is being continually permeated by a stream of fresh solvent and the extracted matter is eventually all concentrated in *A*.

At the end of the extraction (which usually occupies fifteen to thirty minutes) the extract may be recovered by evaporating the solvent in *A*, and may then be measured by appropriate means. To recover the extracted fluid tube *H* is withdrawn from the extraction chamber, washed inside and out, and as much of the supernatant fluid as possible drawn off with a pipette. The remaining solvent may be driven off by warming *C* in a water bath.

Some variants of the above are illustrated in Figs. 2 and 3. In Fig. 1, a 395 mm. West condenser is shown, which experience has proved to be most satisfactory when using ether as a solvent. When solvents of higher boiling point, such as ethyl acetate, are employed, the condenser illustrated in Fig. 2 is adequate, and has the advantage both of lowering the center of gravity of the assembly, and of eliminating one cork joint. This condenser, which may be blown from a test tube, slips loosely into the mouth of the condensation chamber. Under some circumstances, a 15 ml. centrifuge tube may advantageously be used as an extraction chamber (Fig. 3). In this case, the bulb at the end of *H* is omitted, and the tube drawn to a blunt taper. Instead of the concentric sealed joint, *G*, tubes *H* and *F* may pass through separate holes in a cork at the mouth of *D*; however, it is difficult to make a sufficiently tight joint in this way. *A*, *B*, and *C* could easily be equipped with standard taper ground joints in an apparatus intended to be used extensively.

Two illustrative examples of the application of this apparatus may be cited from the author's experience. In attempting to extract quantitatively the lipids from a chylous fluid,⁴ using an alcohol-ether mixture, a stubborn emulsion was encountered upon shaking. Using the apparatus illustrated in Fig. 1, the extraction was accomplished in fifteen minutes without difficulty. In decomposing the picrates of the guanidine bases, the modification shown in Fig. 3 has been especially useful, and the method lends itself to the decomposition of other similar basic derivatives. The picrate was precipitated in 15 ml. centrifuge tubes, and centrifuged. The mother liquor was decanted and the tubes allowed to drain. Then 5 to 7 c.c. of 10 per cent hydrochloric acid was added to the crystals, and the suspension extracted with ethyl acetate for thirty minutes. The base remained as the hydrochloride in the centrifuge tubes, and could be determined manometrically by the hypobromite method. Guanidine, in amounts of 1 to 5 mg., was recovered to the extent of 95 to 98 per cent; and *as*-dimethyl guanidine, in like amounts, gave a uniform recovery of 75 per cent by this method.⁵

SUMMARY

An easily-constructed apparatus is described, which permits the constant extraction of 1 to 10 c.c. of fluid with a lighter, nonmiscible solvent. The apparatus may readily be modified to suit specific requirements.

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COCK CONTROLLED AT A DISTANCE FOR EXPERIMENTAL STUDY OF BREATHING*

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ALL experimental researches on the respiratory gases, basal metabolism determinations, alveolar gas samples, determinations of cardiac output, lung capacity, etc., require the use of a cock with three or more "ways" which gives the patient's mouth communication either with outside air, or with spirometers, gasometers, bags etc.

The cock works by means of a handle attached to it and is turned by the hand of the operator. This is done very close to the patient's face, causing him disturbance which is shown by his altered breathing activity. Moreover, a very

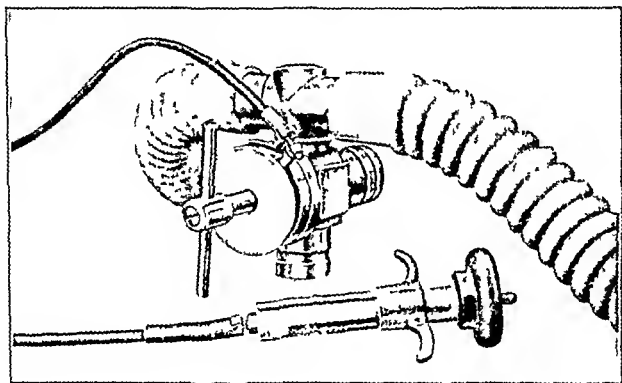


Fig. 1

skillful operator is required in order to watch both the breathing activity of the patient and the stopwatch, besides other experimental particulars.

In order to reduce the work of the operator, I have designed a cock which may be manipulated with remote control (Fig. 1).

It has three outlets. In a fixed position a winding spring connects two outlets. By a turn of the handle the bore of the cock will move through an angle of 90°, one of the outlets will be plugged and the other two will be connected, at the same time the winding spring will be stretched. When the handle is released, the winding spring brings the cock to its first position.

*From the Medical Clinic of the Royal University of Milan.

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This article may be obtained from ING. G. Terzano & C. Milan, Italy.

The first turn of the handle can be executed at a distance by means of a flexible transmitter of Bowden type, which is controlled by a hand stretcher with a stop hook. This keeps the cock permanently in the turned position.

In order to go back to the first position, switch off the stop hook, and the winding spring will automatically turn the handle.

With this device, the operator can place himself beside or behind the patient and work the cock with one hand in a very simple and sure way, leaving it opened afterwards either in one or in the other position for the time required.

CRYSTAL TESTS FOR MINUTE AMOUNTS OF MORPHINE*

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THE reagents to be described provide crystal tests applicable to less than one gamma, one-millionth of a gram of morphine. All are iodine reagents, but they differ in composition and properties and in the crystals they yield with traces of morphine. There are four distinct types of crystals: (1) black needles, (2) rosettes of brown threads, (3) tiny rectangular orange brown rod plates, and (4) much larger red square-cut plates. Six reagents are given; I and II are made with different acids, but yield the same crystals, the black needles. The next two reagents yield the brown threads; with III these vary slightly towards the black needles, and with IV they are associated with the tiny orange brown rectangular crystals. Reagent V yields the tiny rectangular crystals, still associated with some scattered straggling threads; VI yields the familiar red plate crystals, which are commonly considered the most characteristic morphine crystals.

With larger amounts of morphine, in fact, all these reagents yield the plate crystals, where the concentration is suitable; the first three giving chiefly rosettes of brown threads in the outer parts of the test drops where the concentration of morphine is less.

The reagents are not for morphine alone. They give crystals readily with many different amines and alkaloids, and can be used for numerous identifications. Their other uses, however, have not yet been studied; nor do we even know which of the crystals are most characteristic of morphine in testing traces, nor which are formed most readily if the morphine is not perfectly pure.

TECHNIQUE

Add the reagents directly to a little of the dry alkaloid or its salt.

Evaporate a drop of solvent containing morphine on the microscope slide. (Chloroform solutions were used in the investigation—the morphine was dissolved in a little alcohol and dilute solutions made up with chloroform.) Do not allow the drop to spread all over the slide; evaporate it in a small area,

*From the Alcohol Tax Unit, St. Paul Laboratory.
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say by blowing on it. If necessary, evaporate several or any number of drops in the same place. If the amount of morphine suspected is no more than one gamma, draw a line on the slide with the grease pencil pointing to the deposit and ending near it. Add a drop of reagent, then a cover glass ($\frac{3}{4}$ inch), squarely over the deposit. This spreads the reagent evenly and thinly over the deposit and the adjacent slide. Crystallization usually begins in about fifteen minutes, is usually well developed within thirty to forty five minutes, and about as complete as it will ever be in an hour or an hour and a half. The low power of a good microscope is generally adequate, except for the tiny rectangular crystals given by reagents IV and V. These merit examination under high power.

FACTORS CAUSING DIFFERENT EFFECTS OF THE REAGENTS

The reagents are made by diluting specified I KI solutions with diluted or mixed acids. The I KI solutions can be kept in stock, the reagents themselves are not entirely permanent, but a preparation can be used for two or three days, even if left in an open test tube.

The effect of a reagent depends on the ratio of KI to iodine, or the excess of KI over that needed to hold the iodine in solution, and on the acid used. Excess of KI has a solvent effect on the morphine iodine compound, besides changing the crystals from one type to another in the order already mentioned. Hydrochloric and hydrobromic acids slightly increase the solubilities of iodine and of the morphine iodine compound, and acetic acid increases them greatly, sulfuric acid slightly decreases them, and phosphoric acid decreases them to a great extent. Thus with phosphoric acid more KI is needed just to hold the iodine in solution than with the other acids while in spite of the increased KI, the morphine iodine compound is less soluble. Aside from solubilities, hydrochloric acid has a tendency to favor crystallization in black needles, phosphoric acid favors brown threads, and sulfuric acid is more favorable than the others to the formation of plates but is also good for black needles.

One hundred and forty reagent formulas were tested in the experiments. It may be found that the crystals vary with still other factors, particularly temperature. The experiments were carried out at a room temperature of about 22° C.

The following reagents are recommended

<i>Iodine Reagent M I</i> —	I KI solution	Iodine 10 gm
		KI 50 gm
		Water to make 100 cc
	Reagent—Mix 1 cc of the I KI solution with 4 cc of (1 plus 3) H_2SO_4	
	Crystals—Black	needles, more or less branching and in thin rosettes
		Good crystals are obtained with 0.1 gamma
<i>Iodine Reagent M II</i> —	I KI solution	Iodine 5 gm
		KI 30 gm
		Water to make 100 cc
	Reagent—Mix 2 cc of the I KI solution with 3 cc concentrated HCl and 3 cc syrupy H_3PO_4	
	Crystals—Black	needles, mostly in thin rosettes. Good crystals are obtained with 0.1 gamma

This reagent possibly gives the most rapid crystallization, closely followed by reagents I and III.

Iodine Reagent M-III.—I-KI solution: Iodine 7.5 gm.

KI 75.0 gm.

Water to make 100 c.c.

Reagent—Mix 1 c.c. of the I-KI solution with 1.5 c.c. water and 2.5 c.c. syrupy H_2PO_4 .

Crystals—Dark brown threads in rosettes, varying to black needles. Good crystals are obtained with 0.1 gamma.

Iodine Reagent M-IV.—I-KI solution: Iodine 5 gm.

KI 80 gm.

Water to make 100 c.c.

Reagent—Mix 1.5 c.c. of the I-KI solution with 2.5 c.c. water and 3.5 c.c. syrupy H_2PO_4 .

Crystals—Light brown threads in rosettes, interspersed with a few small orange brown rectangular rod plates. The latter become larger and more numerous on standing. Crystals with 0.25 gamma.

Iodine Reagent M-V.—I-KI solution: Iodine 7.3 gm.

KI 60.0 gm.

Water to make 100 c.c.

Reagent—Mix 1 c.c. of the I-KI solution with 4 c.c. of (1 plus 3) H_2SO_4 .

Crystals—A few straggling brown threads, and many tiny rectangular rod plates, mostly brownish orange to orange brown under high power. Generally some of the threads form first; on standing most of the crystallization is in the form of the tiny rectangular crystals. Crystals form with 0.25 gamma.

As an alternative, this reagent can be made with the I-KI solution given for III, mixing 1 c.c. with 4 c.c. of (5 plus 11) H_2SO_4 . Decant from the $KHSO_4$ that gradually crystallizes out.

Iodine Reagent M-VI.—I-KI solution: Iodine 5 gm.

KI 80 gm.

Water to make 100 c.c.

Reagent—Mix 1 c.c. of the I-KI solution with 4 c.c. of (5 plus 11) H_2SO_4 . Decant from the $KHSO_4$ that gradually crystallizes out.

Crystals—The square-cut deep red plates which we generally consider the characteristic morphine-iodine crystals. The color may vary to orange brown. Crystals form with 0.5 gamma; they are of good size with as little as 1 gamma. These crystals require a longer time to form in any number than those with the preceding reagents.

IDENTIFICATION OF TRACES OF MORPHINE

Heretofore the most sensitive identity test known for morphine has been the one with Buckingham's reagent (10 to 15 gm. ammonium or sodium molybdate in 100 c.c. concentrated H_2SO_4 , a permanent reagent as long as it is protected from the moisture of the air). This reagent, a variety of Fröhde's, gives an intense purplish red color which, after a short time, fades out. In a previous

comparative study of the tests for morphine the writer found that Buckingham's reagent gives an unmistakable reaction with 0.4 gamma. As a check on the sensitivity of the new crystal tests, the sensitivity with a single drop of Buckingham's reagent was redetermined using the same solutions used to give deposits of morphine for the crystal tests. In these tests, 0.3 gamma gave a purplish pink, 0.2 gamma a "doubtful pinkish color, 0.1 gamma a negative reaction, or a faint and very doubtful evanescent pinkish color. The crystal tests with reagents I or II, and III, are, therefore, the most sensitive identity tests now known for morphine, and the crystal tests with reagents IV and V are at least as sensitive as Buckingham's color test. Crystallization is perhaps more likely to fail from unknown or uncontrollable factors, but affords a more definite, less subjective test than a color.

It is now possible by two entirely independent tests to identify as little as one gamma of morphine, or even less, provided it can be isolated in two small deposits one on the spot plate and one on the microscope slide. One of these tests, with Buckingham's reagent, is a characteristic color test for a particular phenol—morphine, the other, with one of the iodine reagents given herein, is a characteristic crystal test for a particular amine—again morphine. The identification of enormously larger quantities of most substances is in general based on tests less independent of each other, less characteristic, and less certain than these that identify morphine in the amount of a millionth of a gram.

FROHDE'S REAGENT

A REAGENT FOR MORPHINE AND FOR OTHER PHENOLIC COMPOUNDS*

CHARLES C. FULTON, St. Paul, Minn.

FROHDE introduced his reagent as a test for morphine in 1866, but in spite of its great importance and the long lapse of time, completely accurate accounts of the reagent itself, its morphine reaction, and its effect on other compounds have been entirely lacking.

The reagent is molybdate in concentrated sulfuric acid. It is made by dissolving about 0.5 gm. sodium or ammonium molybdate in 100 cc. concentrated sulfuric acid. More than 1 gm. per 100 cc. should not be used, except for the definite variation, "Buckingham's reagent." As little as 0.1 gm. (1 mg. per cubic centimeter) is sometimes used. Solution is readily effected by warming the acid on the water bath, in fact it can even be obtained at room temperature. Molybdic anhydride will not dissolve so readily. Its solution, however, can be effected by digesting at about 160° C., and it then gives the same reactions.

*From the Alcohol Tax Unit, St. Paul Laboratory.
Received for publication Aug. 9, 1937.

MORPHINE

Added to a little morphine (the free alkaloid or the sulfate) on the spot plate, Fröhde's reagent gives an intense bright purplish red color, after a little time (about four minutes) fading and passing through a comparatively weak brown or brownish, or with a very small quantity of morphine even fading out completely, then developing a strong bright green. On the spot plate this color ordinarily changes on standing to an olivaceous yellow brown or brown yellow. This is due to absorption of moisture from the air. With several cubic centimeters in a test tube, the green solution, in the course of about two hours, becomes a pure deep blue. In a hot dry atmosphere, or if set in a desiccator, the spot-plate test may show, for a time, a bluish green to bluish slate color.

The reaction may be developed by heat. Ordinarily this is inadvisable, for impurities are more likely to react, or if they do not react with the molybdate, may be charred or otherwise decomposed by the hot sulfuric acid, spoiling or obscuring the true colors of the reaction. However, heating may sometimes be of value in testing traces. After adding a drop or two of the reagent, the spot plate is immediately set on the boiling water-bath. The purplish red color quickly fades and passes into the green, and with further heating is changed to slate blue. More heating may change the color to brown; this takes place more quickly the greater the amount of morphine.

Fröhde's reagent is very sensitive. A single drop, applied to a small dry deposit of morphine, strikes a noticeable purplish pink, quickly fading, with 0.001 mg. of morphine. In a dry atmosphere, the green color will develop with 0.003 mg. In the heating test the three colors, purplish pink, green, and slate blue are obtained with 0.002 mg. In a very humid atmosphere, the reagent may absorb moisture too rapidly to show the green color with only a minute trace of morphine. However, under the most adverse conditions, stirring the alkaloid into solution in a "spot" full of reagent, the green color develops throughout the solution within a few minutes of the time the red fades out, with no more than 0.02 mg.

With sulfuric acid that is not quite full strength, the initial color is slightly bluer, varying from purple red to reddish purple.

When made with fuming acid (15 per cent free SO_3), the reagent strikes dark red, which fades almost instantly; the solution becomes colorless or slightly brownish; a green soon develops, and changes to dirty brown. "Fuming Fröhde's" is an extremely valuable reagent for the opium alkaloids in general, although the morphine reaction is not improved by its use.

With the hydrochloride of morphine, Fröhde's reagent strikes a violet color (accompanying the effervescence of HCl); but this quickly changes to the same purplish red given by the free alkaloid or the sulfate, acetate, etc. The color then passes through a weak brown to an olivaceous green, which persists, even in the test tube, as an olive green, long after the sulfate solution (in the test tube) has turned blue.

Heroin reacts like morphine; eodeine, dionin, and papaverine give feeble reactions, green changing to blue, with the ordinary Fröhde's reagent. The

statement met with in some texts that papaverine reacts like morphine is entirely contrary to the observations of the writer. The "Fuming Frohde's" reagent does, however, give a test of great value, an intense violet color, with papaverine.

DESCRIPTIONS IN THE TEXTS

Frohde's morphine test is one of the best and most characteristic known, but the errors, myths, and misdescriptions that fill the texts concerning it are as remarkable as it is.

First, one is usually directed to make the reagent up fresh, and the statement is frequently made that it will not keep. Frohde's reagent is just as permanent as the sulfuric acid from which it is made. In a glass stoppered bottle, or even a rubber bulb dropping flask, it will keep indefinitely, but exposed to the air, the acid absorbs water and becomes diluted and worthless.

Second, the myth persists, and is found in most texts, that the colors are due merely to "lower oxides" of molybdenum. That molybdenum enters into the colored compounds is probable, but that the colors are due solely to the reagent and not to the substance tested is quite impossible. The Peterson, Humes, and Webster Toxicology, as an exception, does have the following as a footnote: "The play of colors obtained by the action of morphine upon the molybdic acid, under the anhydration of the sulfuric acid, is so characteristic of a very few organic compounds, that it must be due to the formation of organic derivatives."¹

The conclusion is certain from the almost unlimited variety of colors produced and the nature of the reactive compounds, which are generally of phenolic character (true phenols or ethers of phenols), or aromatic amines.

To be sure if a too concentrated molybdate solution is used, a deep sapphire blue color develops at the edge and gradually pervades the whole solution, and since this occurs not only with morphine but with all the reactive compounds, this particular color is no doubt due to the molybdenum. But it is not likely that this reservation even includes the blue that develops in the test tube, in the reaction as described above.

Third, is the confusion in regard to Buckingham's reagent. Most texts distinguish it from Frohde's as being made with ammonium molybdate instead of sodium molybdate or molybdic acid. This is no distinction at all. Mulliken even makes Buckingham's reagent with less molybdate than Frohde's, but Prescott² and Fieser³ correctly explain the distinction, which is that Buckingham used more molybdate. Buckingham's reagent can be made with sodium molybdate, but ammonium molybdate contains less water of crystallization in proportion, and, therefore, may be a little better.

Fourth, is the description of the reaction. With Frohde's reagent on the spot plate (at room temperature), the color is never a fine blue, and rarely shows any blue or bluish color at all. Nevertheless, the descriptions in the texts, various as they are, chiefly stress a blue color, and even place it before the green, when they mention a green color at all. They also insist upon a "violet" color. This much abused color term is used sometimes for a purple red, magenta,

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(c) That as nonreactive phenols they also list some which, according to the observations on which this article is based, give very strong reactions, these include the cresols, monochlorophenols, phenol, o-cresol, and thymol

BUCKINGHAM'S REAGENT

This contains 10 to 15 gm ammonium molybdate dissolved in 100 cc of pure concentrated sulfuric acid by heating on the water path, and cooled before use. Buckingham originally used 1 gm molybdate in 15 gm H_2SO_4 .³

With morphine this reagent strikes a very dark purple, changing at once, with solution, to intense purplish red, even stronger, and also more persistent, than with Frohde's reagent. After several minutes, this fades and changes to a weaker brown or olive brown. Also a deep blue color develops at the edge and gradually pervades the whole solution. This blue color is due primarily to the reagent and appears to some extent with the reagent alone, as it absorbs moisture from the air. The fine green color that develops with Frohde's reagent is not produced by Buckingham's.

Thus, considering all the colors, the reaction with Buckingham's reagent is much inferior to that with Frohde's. Since, however, the purplish red color is both stronger and more persistent with Buckingham's, it may be preferred in testing minute traces. The sensitivity may be conservatively set at 0.0004 mg (0.4 gamma) with respect to this one characteristic color, using a single drop of reagent.

In any case, Buckingham's reagent is not a useless variation, since with some compounds other than morphine (salicylic acid and aspirin, for instance) the greater concentration of molybdate is necessary to the reaction.

CONCLUSIONS

1 Frohde's reagent provides a sensitive and specific test for morphine, the main color sequence being intense purplish red, changing to weak brown or even fading out completely, then developing as strong bright green.

2 Although the test has been known since 1866, it has nowhere hitherto* been adequately described, and most descriptions of the color sequence are in error. It does not seem possible to attribute the erroneous and varying descriptions given by eminent toxicologists to faulty observation, apparently some unknown impurity has often produced, in greater or less degree, a blue color which sometimes blends with and sometimes dominates the true colors of the reaction.

3 Besides erroneous descriptions of the colors, several mistakes and myths are very common. Many authors are confused in regard to the difference between Frohde's reagent and Buckingham's variation. (Buckingham used a much higher concentration of molybdate.) Myths that the reagent will not keep, that the colors are due merely to "lower oxides" of molybdenum.

4 In obtaining characteristic tests for reactive compounds, the "Fuming Frohde's" (made with fuming H_2SO_4) and Buckingham's reagent are of great value, perhaps equally with the normal reagent.

*The writer's own description without the discussion given here has just been published as one of The Principal Chemical Tests for Morphine. Am J Pharm 109 228 1937.

5. Compounds with which Fröhde's reagent reacts are, in general, phenols or ethers of phenols, or in some cases aromatic amines. All kinds of colors and sequences of colors are produced, and, in consequence, characteristic or specific tests can be obtained for many different compounds.

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CRYPT ASPIRATION: SPRAY CULTURE METHOD FOR THE ISOLATION OF B. DYSENTERIAE

JOSEPH FELSEN, M.D., NEW YORK, N. Y.

DURING the past few years we have been using the following method to advantage in our dysentery work. It is particularly useful in the chronic dysentery cases because recovery of the specific organism by ordinary routine fecal culture methods is usually unsuccessful.

Principle.—By means of a crypt aspirator and sigmoidoscope the material from the deep recesses of the crypts or ulcers is sucked out and sprayed directly upon Endo or other suitable plate medium.

Apparatus.—(1) Sigmoidoscope. Any type will do as long as it provides an adequately illuminated field for securing culture material by direct vision.

(2) Crypt aspirator. A 35 cm. heavy wall capillary glass tube (outside diameter 5.5 mm., bore approximately 0.5 mm., A. H. Thomas catalogue No. 6088) with the distal 2.5 cm. bent to an angle of approximately 20°. Just proximal to the point of angulation there is blown a small bulbous reservoir, sufficient to hold two or three drops of fluid. This is accomplished by sealing the distal end of the dry tube, heating the area to be expanded to a red heat

and blowing through the proximal end until the desired size is obtained. With a little practice angulation of the tip and bulbous reservoir can be made at one operation. The seal at the distal end is broken, and both ends of the tube smoothed off in the flame. An ordinary 20 cc rubber bulb, such as that which comes with therapeutic serum outfits, is used to create suction. The bulb has a

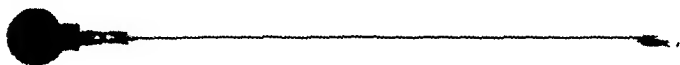


Fig 1—Mucosal aspirator for taking cultures from crypts or from floor of ulcer. Length of glass tube 35 cm (14 inches) sufficient for insertion through a 1 $\frac{1}{2}$ inch sigmoidoscope. Note fusiform dilatation near tip which is bent slightly for better approximation to intestinal wall.

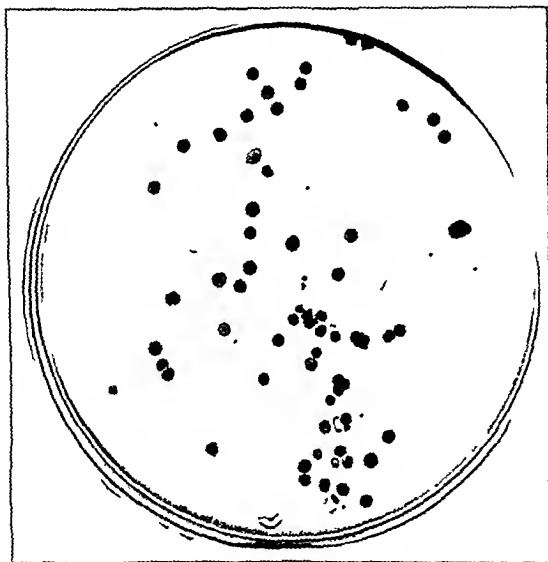


Fig 2—Plate inoculated by spray culture method. Note isolated colonies.

small hole in its dome, so that when it is attached to the proximal end of the aspiration tube it may be compressed without forcing air or fluid out of the tube. Then by placing the index finger over the hole, suction is created by releasing pressure on the bulb.

(3) Culture medium. We still find a properly prepared Endo plate satisfactory, but any suitable substitute may be found equally satisfactory.

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THE CHEMICAL DIAGNOSIS OF PREGNANCY BY DETECTION OF ESTRIN IN URINE*

III MODIFICATIONS OF PROCEDURE AND RESULTS IN 193 DETERMINATIONS

M J SCHMULOVITZ A B † AND H BOYD WILKIN, M D, BALTIMORE, MD

IN 1935 we described a method for the chemical diagnosis of pregnancy based on the detection of estrin in the urine,¹ and reported the results obtained with this method in 89 analyses. Since then we have simplified the procedure in several details of operation and apparatus, and we have done a total of 193 analyses. It is this further work which we wish to report at this time.

It may be well to briefly summarize the original method in order to understand the changes which have been made. The twenty four hour urine was brought to pH 4 with hydrochloric acid and evaporated down to about 200 cc. The urine was then brought to pH 1 with concentrated hydrochloric acid and extracted for sixteen hours with ether in a special apparatus which consisted of a reflux condenser connected with four tubes, one containing the urine to be extracted and three containing carbonate solution to wash the ether extract. (This apparatus has been greatly simplified.) The ether extract was then washed free of all aromatic hydroxy acids with sodium carbonate and, after removing the ether by distillation, the residue was distilled with water until free of all volatile phenols. The last traces of water were removed by drying in a glycerin bath, and the estrin was detected by coupling it with diazotized p nitroaniline to form a deep colored azo dye. The amount of color was estimated as millimeters of standard solution of ferric chloride (33 per cent) necessary to match the color test placed at 10 mm, and this was called the FN (ferric chloride number), an FN over 25 indicating pregnancy.

APPARATUS

The principal modification has been the devising of a much simpler extraction washing apparatus. Whereas our original apparatus required fairly skilled glass blowing in its construction and was difficult to assemble, the present device is assembled from the usual laboratory apparatus and requires but one T sealed glass joint. The first extractor of this type was described by us² in April 1936, and consisted of a urine extraction tube, a sodium carbonate washer, and an ether extraction flask. This has now been simplified

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even further, in that the carbonate washer has been eliminated by placing the sodium carbonate solution in the ether extraction flask. The final assembly is illustrated in Fig. 1.

Except for the sealing of the side arm to the Kjeldahl flask, which requires some experience,² the only other special apparatus is the glass inner tube. This may easily be made from ordinary 6 mm. soft glass tubing by blowing a bulb on the lower end. In order to provide a fine spray of ether, small holes are punched around the bulb with a strand of wire taken from a

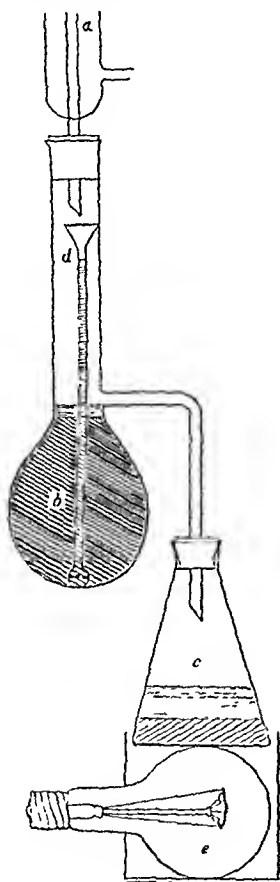


Fig. 1.

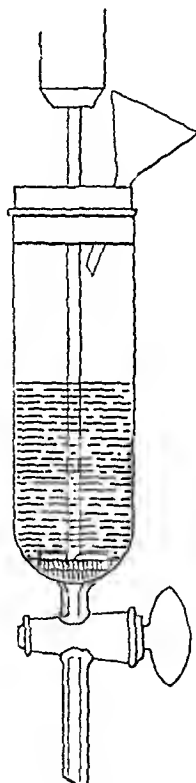


Fig. 2.

Fig. 1.—*a*, Condenser; *b*, 500 c.c. Kjeldahl flask to which is sealed a 11 mm. diameter sidearm as indicated; *c*, 250 c.c. Erlenmeyer flask; *d*, inner tube, 6 mm. diameter, with small holes at lower end; *e*, 100-watt lamp in asbestos lined tin can.

Fig. 2.—Apparatus for washing ether extract.

chromel wire gauze (A. H. Thomas Co., Catalogue No. 9993). This wire is sharpened to a fine point and held in a pliers or sealed into a glass rod. The bulb and pointed wire are heated with a fine pinpoint blow-torch flame, and with a little practice, fine holes are easily punched. The upper end of the inner tube is flanged out with a carbon rod from an arc lamp.

In addition to being more easily constructed, time is saved with this apparatus, since the urine need now be concentrated only to 400 c.c. instead of

*E. J. Callahan & Co., Baltimore, Md., can supply such flasks.

200 cc as formerly, and the apparatus is filled and assembled for operation much more quickly than the original one

Before recommending the placing of carbonate solution below the ether in flask C (Fig 1) experiments were done to determine that no estin was lost in the carbonate Full term pregnancy urines were extracted, the carbonate underlayer was removed and washed twice with small portions of fresh ether which were then added to the main ether fraction The carbonate was made strongly acid with hydrochloric acid and thoroughly extracted with ether The ether residue was transferred to olive oil and injected into castrated rats No estin was demonstrable

Our method of evaporating the twenty four hour specimen is of interest, since it has led to the construction of a simple device which automatically signals with a bell when the evaporation is completed Since such an apparatus is of general interest in many operations where it is desirable to carry out odoriferous evaporations in distant hoods, we are describing it on page 642 of this issue Using this apparatus, we have never lost a urine by accidental burning

Another time saving procedure has been developed in the method of washing the ether extract At the time of our first report this was done by means of the usual separatory funnel Much time and effort can be saved by washing in a cylindrical separatory funnel by means of a motor driven stirrer This is illustrated in Fig 2 The paddle should be placed just at the interface of the two phases, and the direction of rotation of the blades should be such that the carbonate is thrown up into the ether

At the conclusion of the water distillation, the original procedure directed the removal of the last traces of water by heating in a glycerin bath at 160°C This process was time consuming and required frequent attention, for accidental elevation of temperature caused charring and seemed to diminish the red value given by positive tests, giving dark brown tints instead This has been simplified by placing the distilling flask, containing the last 10-15 cc of water in a boiling water bath and removing the water under vacuum, the ordinary laboratory water vacuum pump does this in a few minutes The rubber hose from the pump is slipped directly over the side arm of the distillation flask

PROCEDURE

The procedure is essentially the same as the original, but we should like to amplify more fully a few points which we have found to be sources of error to other workers when they first begin doing the test

The pH of Hydrolysis—At the time our paper¹ appeared it seemed likely from the work of others^{3,4} that an acidity for hydrolysis greater than pH 4 was necessary to obtain a maximum deconjugation of estin esters We pointed this out in a mimeographed supplement, the availability of which was noted at the end of our paper,¹ and we recommended therein the use of pH 1 as possibly giving better yields of free estin and improving the results in the diagnosis of

early pregnancy. We noted that experiments to settle this point were in progress. The results of these experiments concerning the optimum acidity of hydrolysis have been reported in this journal.⁵ Briefly summarized, they showed that after pH 4 hydrolysis and complete extraction of the deconjugated hormone, subsequent hydrolysis at higher acidities does release still more hormone, but the additional yield then obtained is always much lower than that first obtained at pH 4. On the other hand, with strong acid hydrolysis there is produced in urine a compound which is ether-soluble, insoluble in sodium carbonate from ether solution, nonvolatile with steam, and capable of coupling with diazotized p-nitroaniline to form a deep red azo dye; that is, it has all these chemical properties in common with estrin, but *it is not estrogenic*. This compound, which we are tentatively calling "chromogen," is produced in about 7 out of 10 urines when 15 per cent hydrochloric acid hydrolysis (15 c.c. of conc. HCl per 100 c.c. urine) is used and occasionally at pH 1, and is obtained from *any* urine. We have never obtained it at pH 4. While we do not deny that an acidity much greater than pH 4 may be necessary to quantitatively hydrolyze estrin esters, it is seen from this work that in this procedure such acidities may cause false positives due to the formation of chromogen. For accurate results in pregnancy diagnosis one must, therefore, use pH 4 hydrolysis as originally directed until the nature of the chromogen can be determined and a method for its removal devised.

After hydrolysis at pH 4, we suggested that the urine be adjusted to pH 1 before extraction, since there seemed to be evidence in the literature^{6,7} that extraction was more efficient at the latter concentration. From further work we now know that making the urine strongly acid at room temperature produces neither chromogen nor estrin deconjugation; boiling of the strongly acidified urine is necessary to obtain this. This is in agreement with the finding of Marrian³ that high acidities at low temperatures produce no significant hydrolysis of estrin esters. The adjustment to pH 1 is therefore omitted.

The washing of the ether extract with sodium carbonate has been modified only in the fact that the saturated carbonate solution is prepared at the time of use, rather than preparing a large volume of stock solution as originally directed. The reason for this is that such a solution (sp. gr. 1.22 at 23° C.) will not remain saturated under the usual variations in laboratory temperature, especially in cold weather. It is simpler to stir an excess of carbonate with water just before use. We would stress at this point that this washing must be continued until the carbonate wash gives a negative test with 1 c.c. of the color reagent. This is best determined by comparison with a control made of an equal amount of carbonate solution and 1 c.c. of reagent. The two colors should be compared by looking through the length of the liquid column against a white background. Failure to wash completely will give an increased amount of false color in the final test. In the usual determination, 20 to 30 carbonate washes are required. We have observed that when the washing occasionally seems to progress very slowly that the interjection of acid washes seems to hasten the process. Acid washes are therefore used until they no longer show any change on testing with the reagent.

The acid washes (2 per cent HCl) are saturated with sodium chloride and are tested by adding 1 c.c. of color reagent and making alkaline with sodium carbonate solution. The acid washes and a final wash of distilled water always conclude the washing procedure, this is done because phenol is not volatile from strongly alkaline solution, and when ether is distilled off after prolonged washing with aqueous carbonate, there remains a fair amount of white residue, presumably carbonate.

The same precaution to insure completeness is to be stressed in the phenol distillation. Here, as above, the color given by distillate, 1 c.c. of color reagent and carbonate solution must match exactly a control made at the same time with distilled water. Incomplete distillation seems to be the most common source of error made by those first working with the procedure. The distillation is best conducted from a 500 c.c. distilling flask, the water level being maintained at between 150 to 300 c.c. The amount of distillate required before a truly negative test is obtained is usually about 700 to 1,000 c.c. Fresh corks in the distilling apparatus give markedly positive color tests in the distillate. This factor is completely eliminated by placing the delivery tube of the distilling flask well into the condenser without a cork and by closing the mouth of the flask with any loose fitting glass stopper, the condensed vapors seal it very efficiently.

No change has been made in the technique of the final color test nor the method of ferric chloride comparison. Many workers have told us that they experience difficulty in comparing the predominantly red color of strongly positive tests with the predominantly brown color of ferric chloride. This is quite true, and the comparison is more a balance of density rather than a true color matching, the ferric chloride does match the yellow brown colors of negative tests. To obviate this difficulty, we have found that beta naphthol solutions when coupled give a predominantly red dye which matches very well the red colors of positive tests. The beta naphthol standard contains 0.2 mg. beta naphthol in 8 c.c. of 95 per cent ethyl alcohol. This is made from 2 c.c. of a dilute stock standard, containing 0.1 mg. per c.c. by the addition of 6 c.c. of alcohol. To this is added 1 c.c. of the color reagent, and then 1 c.c. of 10 per cent sodium hydroxide. The reason for using sodium hydroxide here is that when beta naphthol is coupled in sodium carbonate solution by the pregnancy test technique, it gives yellow orange rather than red colors. The BN (beta naphthol number) is obtained exactly as the FN and can be converted to the FN by multiplying it by 5.97. The use of beta naphthol is optional. While the 33 per cent ferric chloride solution reaches a color on standing which is quite stable for many months as checked by tintometer readings, beta naphthol solutions decrease in tintometric value in several weeks.

In the interpretation of the final color test we have observed that the presence of a red tint which tends to stain the filter paper is very suggestive of increased estriol. Further experience would indicate that the FN interpretation as previously given, negative below 15, doubtful between 15 to 25, and

positive over 25, should be shifted to 20, 20 to 30, and over 30, respectively. While the limits of these readings apply to the vast majority of urines, one will occasionally encounter a negative urine, the final alcohol extract of which contains so much brown pigment (the color of which does not increase appreciably on coupling) that the FN will be about 25, but the test will be a pure brown without a trace of red. In most of the tests, however, no difficulty is encountered, since pregnancies as early as the fourth week often give an FN of 50 with markedly red color tests. In suspected earlier cases with an FN of 30, for example, one does not feel certain and prefers to do another test at a later date.

RESULTS

At this writing we have done 193 urinalyses by this method. The results are summarized in Table I.

TABLE I

	NO.	PER CENT
True positive results	99	51.3
True negative results	44	22.8
Doubtful results	19	9.9
False positive results	17	8.8
False negative results	7	3.6
Subsequent history could not be obtained	7	3.6
	<u>193</u>	<u>100.0</u>

Only those tests are designated as true positives or true negatives which subsequent clinical study over a period of months or longer corroborated. Almost all of the cases in our series, after the first few which we ran, were difficult problem cases; they all had gynecologic or obstetrical complications, some endocrinopathy, or a previous history which made the clinical diagnosis uncertain. The routine type of case, in which a pregnancy test is done because of a suggestive history and the absence of definite physical signs of pregnancy, was not accepted in this series. Doubtful results (results showing intermediate quantities of estrin excretion) obtained in conditions other than pregnancy are generally significant in that they are associated with some endocrine disturbance. In such cases, of course, later tests do not show the rising estrin excretion which is characteristic of pregnancy. Our 19 doubtful tests included the following conditions:

- 4 Threatened abortion.
- 3 Less than four weeks pregnant; confirmed by later tests.
- 3 Teratoma testis.
- 2 Irregular menses and pelvic mass.
- 1 Missed abortion.
- 1 Fetal skeleton in abdominal cavity for three years.
- 1 Eleven to twelve weeks pregnant.
- 1 Ten days postpartum.
- 1 Menses delayed two weeks—early abortion (!).
- 1 Contracted pelvis: three to four months pregnant, 2 Friedman tests negative, estrin bioassay doubtful.
- 1 Marked obesity; six months pregnant.

Of our 17 false positive results, a few may have been due to technical errors, but, on the whole, they probably represent conditions in which for pathologic reasons at present unknown there is actually an increased estinuria. They were distributed as follows:

- 4 Theelin administered in large dosage before specimen was collected
- 5 Amenorrhea—origin undetermined, clinically not pregnant
- 1 Six week menstrual cycle. This specimen measured 4,760 c.c. (48 hours') and was passed just before the menses (normal peak of urinary estin)
- 1 Ruptured ectopic pregnancy
- 1 Nine year old female—endocrinologist's examination in hospital history quoted: "Hypertrichosis—pubic and general. Breasts well developed and large areolae. She has certain signs and symptoms suggestive of hyperadrenialism. Premature appearance of secondary sex characteristics. Possibly basophilic adenoma of pituitary and secondary hyperplasia of adrenal cortex."
- 1 Pituitary dysfunction, diabetes insipidus, multiple xanthomatosis, II and Schuller-Christian disease (?) Fourteen year old female: amenorrhea, obesity, polydipsia, polyuria
- 1 Hypopituitarism (?) Twenty seven year old female, abnormal hair distribution, occipital headaches, irregular menses, laparotomy—adrenals normal, ovary prolapsed
- 1 Uterine fibroid
- 2 One patient middle aged male. Testicular swelling decreasing in size on x-ray irradiation, followed by operative removal. Pathologic diagnosis—inflammatory disease. Clinical diagnosis—teratoma testis

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The case listed above of possible hyperadrenialism is of interest since Saphin and Parker⁸ have also reported increased urinary estin in a case of hyperadrenialism.

False negative results were obtained in the following 7 analyses:

- 1 Four days past expected menstrual period—ten days later gave positive test
- 1 Three weeks past expected menses, Friedman test positive
- 1 Pregnancy with marked obesity, also gave 1 doubtful test
- 1 Clinically eight weeks pregnant at four weeks gave positive test
- 3 One patient—constantly denied sexual intercourse; pregnancy confirmed by x-ray

7

The last case listed gave 3 negative tests at about the second, third, and fourth months of pregnancy. She vigorously denied any possibility of being pregnant and desired operative interference. Pregnancy was verified at about the fifth month by x-ray. (We believe that any patient capable of such persistent prevarication is also quite apt to substitute urine of some other person in her efforts to obtain operative intervention.) Crew,^{9,10} in a very wide experience with Aschheim Zondek and Friedman tests, explains somewhat similar results as due to psychic inhibition of hormone excretion.

The conditions listed above which gave false positive or doubtful results did not do so uniformly. Correct results were obtained in cases of teratoma testis, ovarian cyst, fibroids, missed abortions, diabetes insipidus, ectopic pregnancy, and "endocrinopathies"—including pituitary dyscrasias. We have observed in several cases that when bleeding complicates pregnancy, the urinary estin is much lower than normal.

DISCUSSION OF RESULTS

While an incidence of 8.8 per cent of false positive results may seem high in comparison with the Friedman test, which is commonly stated to give an accuracy of 98 per cent, we would point out that when prolan tests are done on a series of *problem* cases such as this, the incidence of false positive results is just as high or higher. This fact has been demonstrated by many workers¹¹⁻¹⁵ and, we feel, it is not sufficiently well recognized by those who write the usual textbook figures of 98 per cent accuracy for Friedman tests without any qualification.

In our present series of cases, one patient, two months pregnant, gave a negative Friedman test and positive chemical test. Another in which the date of intercourse was known, gave a negative Friedman and positive chemical test at three and a half weeks of pregnancy; the Friedman test repeated on the same rabbits at the seventh week was positive. While our number of such cases is certainly too small to say that the chemical test is as reliable as the Friedman test in early pregnancy, this may be the case. In this connection it should be pointed out that Browne and Venning¹⁶ and Evans and his associates¹⁷ have recently upset the hitherto long accepted belief in a very high prolan excretion in the "first weeks" of pregnancy by demonstrating "the invariable existence of an exceedingly steep and high hormone peak at a time which is quite accurately one month from the beginning of the first expected but missed menstruation" (quoting Evans). It is possible that false negative prolan tests in early pregnancy are to be explained by this peak not yet having been reached.

One of the great advantages of chemical estimation of estrin by the colorimetric method, as contrasted to the biological pregnancy tests, is that a relatively quantitative estimation of hormone excretion is obtained. This quality of the procedure has been utilized by Savage and Wylie¹⁸ in studying the differences in estrin excretion in normal pregnancy, preeclampsia, and chronic nephritis complicating pregnancy.

OTHER CHEMICAL REACTIONS FOR ESTRIN

We have made many attempts to shorten or modify our procedure, or to find some other reaction which could be carried out very rapidly, but so far these efforts have been without success.

The meta-dinitrobenzene reaction¹⁹ of theelin, the silico-tungstic acid reaction,²⁰ and the concentrated sulfuric acid reaction²¹ of theelin and theelin in our experiments have not been sufficiently sensitive to react with quantities of hormone demonstrable with the diazonium reagent. The trichloroacetic acid reaction, and the concentrated sulfuric acid—acetic anhydride reaction of sterols gave negative results with quantities of hormone extracts which gave strongly positive color reactions with the diazonium reagent.

The simple chemical pregnancy test of Visscher and Bowman²² (not a test for estrin) has not shown in our hands any difference in reaction between normal male urines and those of advanced pregnancy.

We have attempted to eliminate the process of ether extraction by evaporating the urine to dryness and leaching the residue with ether but could obtain no estrin in this manner, although equal fractions of the same urine gave positive reactions by our routine procedure and by extraction and bioassay.

The Marrian method of titometric estimation³ of theelin and theelol with phenolsulfonic acid has given us good results with late pregnancy urine, we have thus far found it unsatisfactory for demonstrating estrin in early pregnancy.

With the appearance of the tungstic acid method of precipitating urinary estrin,²¹ we thought at first that this method might eliminate our long extraction of estrin with ether. However, our results with this method of extraction have uniformly given excessively high amounts of color on normal male urines.

We have recently found that norite (activated charcoal) quantitatively adsorbs estrin from pregnancy urine. Conjugated estrin seems to be adsorbed as well as free estrin. Several experiments indicate a very efficient elution of the norite adsorbed estrin by butyl alcohol. The much smaller bulk of norite precipitate and the simplicity of this elution as contrasted to the elution in the tungstic acid method make this method worthy of further study in an effort to simplify the rapid removal of estrin from urine.

SUMMARY

Modifications and improvements in a method for the chemical detection of urinary estrin are described. Results obtained with this method in 193 determinations made mostly on problem cases are presented and discussed. Results obtained in attempts to diagnose pregnancy by other rapid chemical methods are given.

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AN AUTOMATIC EVAPORATION CONTROLLER AND SIGNAL*

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ALTHOUGH open evaporation by heat is a useful laboratory practice, the procedure involves repeated inspections in order to control the degree of concentration, or to prevent damage to some valuable biological fluid or organic solution. This supervision is tiresome and time-consuming, especially if the evaporation is going on at some distance from the operator. Losses often occur, both of material and apparatus, because of forgetfulness, procrastination or interference.

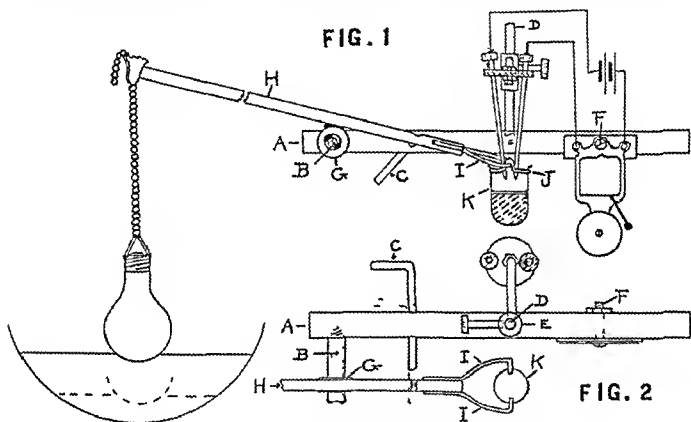
After a few discouraging experiences with urine evaporations coincident to hydrolysis of estrin-esters for use in a chemical test for pregnancy,¹ the author devised a simple apparatus which gives a bell signal when the evaporating liquid reaches any predetermined volume or level.

Because the device has proved to be reliable over a period of two years, a brief description is given in the belief that it may be of value to other workers.

Description.—Fig. 1 shows the front view of the apparatus when clamped in operative position. Fig. 2 shows the top plan, with the electric contact assembly swung 180° out of its usual position on *D* in order to illustrate the shapes of the trunnion arms *I-I* which support *J* and *K*, Fig. 1. All metal parts are of brass. The figures are not to scale.

*From the Department of Biological Chemistry, University of Maryland, School of Medicine.

A is a 12 inch piece $\frac{1}{2}$ inch rod, B is $\frac{1}{16}$ inch rod, $2\frac{1}{4}$ inches long, which is threaded or sweated in A at $\frac{1}{2}$ inch from its end, C is $\frac{1}{8}$ inch rod, $4\frac{1}{2}$ inches long bent as shown, and fits loosely in a hole drilled through A at $2\frac{1}{2}$ inches from B , and parallel to B , D is $\frac{1}{16}$ inch rod, $3\frac{3}{4}$ inches long, and is threaded into A at $5\frac{1}{2}$ inches from B and at right angles to B . D supports the electrical contact assembly through a heavy collar E , Fig 2. The latter slides up and down on D and is fitted with an adjusting set screw and a $\frac{1}{16}$ -inch support. This support is bent and threaded for clamping the $1\frac{1}{4}$ inch circular or square insulating block, $\frac{1}{4}$ inch thick, in which the two converging contacts or electrodes are held tightly by friction. The electrodes, 4 inches over all, are made of $\frac{1}{8}$ inch rod. They are fitted with knurled nuts at the top and are pointed at the lower ends which center on K when in place. F is a bolt through A which clamps in electric bell in place against a piece of flat insulating fiber



Figs 1 and 2

Returning to the left end again, G is a washer $\frac{1}{2}$ inch in diameter, $\frac{1}{4}$ inch thick, and with a $\frac{1}{16}$ inch hole. It is fitted to rotate freely on B . G is held loosely in place by collars on B , the outer shorter collar is soldered to the free end of B . G is spaced $1\frac{1}{2}$ inches from A . H is a piece of $\frac{1}{16}$ inch rod, 12 to 16 inches long. It is soldered to G at a point 4 inches from its right end as shown in Fig 1. I are made of $\frac{1}{16}$ inch wire, soldered to H as shown in Fig 2, and shaped to act as tinnies which suspend the collar J at $1\frac{1}{2}$ inches from the right end of H (Fig 2). J is a collar made of $\frac{1}{16}$ inch wire. It is formed to suspend counterweight tube K which is made from the lower $1\frac{1}{2}$ inches of a 1 inch diameter test tube (or a small beaker). K is flared at the outlet to retain J in position, K should swing freely in its fittings.

On the left end of H a split bell from a pull chain is soldered in an inverted position, with the split away from the end of H . Into the bell is adjusted the desired length (12 to 16 inches) of pull chain. To the lower free end of this chain the apex of a V shaped piece of $\frac{1}{16}$ inch wire is soldered, which in turn is soldered as shown to the base of a 25 watt frosted inside electric bulb.

The whole apparatus may be of simpler design and yet be useful. For example, *A* may be a piece of wood, provided with a metal extension for clamping to a stand. *B* and *D* may be nails, and *G* a brass battery nut. Ordinary insulated wires may be wound around *D* forming electrodes at their free ends. *H* may be a cheap curtain rod, split at the right end to encircle and support *K*, thus omitting *I-I* and *J*. The bell is screwed to the wood body of the apparatus. This simple arrangement may be assembled from materials in any laboratory. In fact, our first controller was built in this way and is still dependable.

Operation.—Clamp the right end of *A* to a laboratory stand. Wire the bell and electrodes in series with two 6-inch dry cells, as shown. (The bell may be wired to the operator's laboratory if more convenient. In this case use three dry cells.) *Do not use a switch.*

With the float bulb hanging freely, and stop *C* pulled out of the way, add clean metallic mercury to counterweight tube *K*, until the bulb is in balance. Add a $\frac{1}{16}$ -inch layer of clean mineral oil to the surface of the mercury.

Place two or three pieces of chromel wire gauze on a large tripod. This is important for heat distribution from a large flame, as from a quadruple burner.

Prepare an apron, 16 inches square of 4 to 6 thicknesses of asbestos paper clamped together by a paper-stapling machine. Cut a 4- to 6-inch center opening and place this over the tripod. (This apron prevents charring of the contents of the receding liquid as it dries on the walls of the container.)

Now raise the float beam *H* at the left end and hold here by stop *C*.

Place a porcelain dish on the prepared tripod and press it firmly into place. Measure into the dish the predetermined volume at which you wish the apparatus to give a signal. Now release the beam and adjust the weight of mercury in the counterweight tube and the length of the suspension chain, so that the rounded portion of the bulb will sink about $\frac{3}{8}$ -inch below the surface at this level.

Adjust the electrodes coarsely with the sliding collar, and delicately by rotating one electrode in the insulating block, so that the bell rings at this point. Pour in the remainder of the liquid; this raises the bulb. The bell stops ringing. Raise the bulb again about 3 inches. It should fall to the surface at the higher liquid-level from this point.

Light the burner and forget the evaporation—until the bell rings again.

Advantages.—This simple controller has been used to control hundreds of evaporations without a single loss of apparatus or material.

The electrical contacts are dependable; they are sensitive and do not corrode.

The apparatus may be used to control the evaporation of very large or small quantities of liquids.

With the large 30-liter dishes the controller is effective down to a level where the final liquid depth is about 20 mm.

Using smaller porcelain dishes, it is possible to evaporate 4,000 c.c. to 100 c.c., or by using a 200 mm. dish, to evaporate 300 to 400 c.c. to 25 c.c. with safety.

NOTE.—The author has devised a more complicated apparatus which will begin to turn down the gas flame at any desired stage of the evaporation, then will simultaneously turn off the gas entirely and give a bell signal at its completion. The author will be glad to send line drawings of this more complex apparatus to interested investigators.

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A SIMPLE PROTECTION IN ASEPTIC GRINDING OF TISSUE

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WHEN grinding tissue in a mortar, it has been found that the following device aids materially in promoting asepsis, as well as protecting the operator from splashed droplets or bits of sand.

This device consists of an apron of paper toweling, cloth or gauze, fitted loosely over the mortar and tied firmly to the pestle with string. This apron



Fig. 1

is made of a straight piece of paper or other material about one and a half times the circumference of the mortar, and long enough to come well over the edge of the mortar. It is fastened to the pestle by gathering it in and tying with a piece of string. The apparatus with the apron in place is wrapped and sterilized as usual. At the time of use, the edge of the paper can be folded up. This forms an umbrella-like shield which protects the contents of the mortar from external contamination during grinding.

A TIMESAVING METHOD FOR EXAMINING BLOOD FILMS WITH THE HIGH DRY (45x) AND OIL IMMERSION OBJECTIVES*

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THE advantage of this method is that a large surface of the slide can be examined in less time than with the oil immersion objective. The effect is as brilliant as though the smear were permanently mounted under a cover glass.

The method consists of spreading a thin even film of immersion oil over the entire slide. A straight piece of glass rod or tubing is best for spreading the oil. If it is uneven, permitting the slide to stand on the desk a few minutes will remedy this. The film of oil should be thin enough to permit focusing

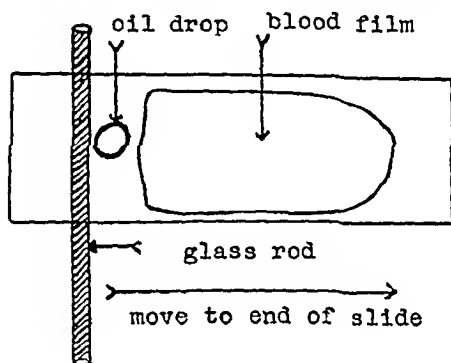


Fig. 1.

with the high dry lens without getting oil on it. Doubtful or interesting cells can be studied under the oil immersion objective simply by rotating the nose-piece.

FURTHER OBSERVATIONS ON THE USE OF NIGROSINE TO DEMONSTRATE *TREPONEMA PALLIDUM* IN SYPHILITIC LESIONS†

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THE detection of *T. pallidum* in lesions is of great diagnostic value in doubtful cases of syphilis and a factor of considerable importance in the relation to an early diagnosis.

A method for the demonstration of *T. pallidum* in syphilitic lesions was recently described by Dienst and Sanderson. *Treponema pallidum* is difficult to stain with the dyes ordinarily used in bacteriologic examination, consequently its characteristic morphology in exudates can best be demonstrated by dark-

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field illumination or by some method which stains the background and leaves the spirochetes and other organisms colorless. Nigrosine was found to be far superior to any other agent used for this particular purpose.

PREPARATION OF STAIN

The stain is prepared by dissolving 5 grams of nigrosine (Coleman and Bell) in 100 cc of distilled water. This is accomplished more easily by heating the mixture in an Erlenmeyer flask in boiling water for thirty minutes. Heating in the autoclave at 15 pounds pressure for fifteen minutes is equally satisfactory. One half a cubic centimeter of 37 per cent formalin is added as a preservative. The stain should be filtered before being used.

METHOD OF STAINING

Use the ordinary bacteriologic inoculating needle and take one small loopful of the exudate from the freshly cleansed lesion and place it in a small loopful

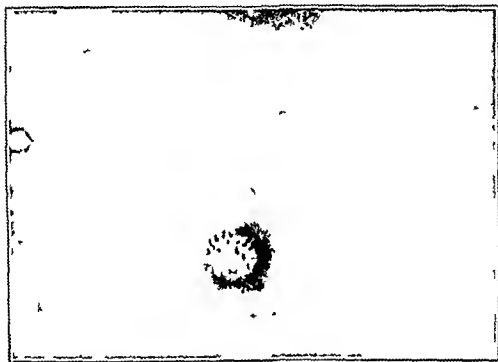


Fig 1—*T. pallidum* from a syphilitic lesion as demonstrated by the use of nigrosine ($\times 2500$)

of water on a clean slide. Then add a loopful of 5 per cent nigrosine and mix rapidly by rotary movements, then gradually increase the size of the smear by spreading out from the center. Satisfactory thinness of the preparation is obtained in this manner. Place a drop of immersion oil directly on the smear and examine for spirochetes, using a good substage lamp for sufficient lighting. The *T. pallidum* if present will appear as finely coiled, colorless organisms, and the remainder of the area will be stained gray to blue black, depending on the thickness of the preparation. A very sharp focus is necessary to visualize the spirochete of syphilis because of the delicateness of its structure (Fig 1). When the preparation is properly made, the delicate terminal filaments of the spirochete are sometimes visible by this method.

Very satisfactory results have been obtained from exudative material after it has dried on the slide. In this case a loopful of water is placed on the area of the dried exudate and the material dissolved. Then a loopful of the dissolved exudate is placed on a clean slide and a loopful of 5 per cent nigrosine is added. Smear, dry, and examine in the same manner as for the fresh exudate.

RESULTS

Exudates were examined from 50 patients with the following results: Thirty-six patients were diagnosed positive either by the dark-field illumination test or by serologic blood tests, and thirty-five exudates from these same thirty-six patients were found to contain *T. pallidum* by the nigrosine preparation. One particular specimen was diagnosed positive by the dark-field test, but the spirochetes seen in the nigrosine preparation were not morphologically typical of *T. pallidum*.

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THE DETERMINATION OF SULFANILAMIDE IN CEREBROSPINAL
FLUID BY MEANS OF SODIUM-BETA-NAPHTHOQUINONE-4-
SULFONATE*

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RECENT experimental and clinical studies with sulfanilamide show that this drug is a very powerful chemotherapeutic anti-infective agent.¹⁻⁵ Marshall and his associates have devised very good methods for the determination of the sulfanilamide content of alcoholic⁶ and toluenesulfonic acid⁷ filtrates of blood and other body fluids. In an extended investigation of the reaction between sodium-beta-naphthoquinone-4-sulfonate and various amino compounds,⁸ information was secured which led to the development of a quantitative method for the determination of the sulfanilamide content of tungstic acid blood filtrates.⁹ A direct method for cerebrospinal fluid analysis is now offered which utilizes the same reagent but eliminates filtrate preparation.

EXPERIMENTAL

The spinal fluids were obtained from the University Hospital laboratory through the kindness and cooperation of Miss B. Jahns. Although cerebrospinal fluid contains many compounds, such as amino acids, amines, ammonia, etc., which react with the beta-naphthoquinonesulfonic acid in slightly alkaline solution, no reaction or color intensification occurs with this reagent if the spinal fluid is first made slightly acid. In our previous work,⁸ however, it was found that certain aromatic compounds like sulfanilamide, sulfanilic acid, anisidine, aminophenol, etc., gave colored solutions or precipitates with the chromogenic reagent even in acid medium. This fact permits the determination of sulfanilamide in blood and cerebrospinal fluid despite the presence of compounds which normally react with the quinone reagent.

*From the Department of Biological Chemistry, University of Maryland, School of Medicine, Baltimore.

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One cubic centimeter of spinal fluid is diluted to 10 cc with distilled water. Ten cubic centimeter quantities of various standards containing 0.01 to 0.20 mg sulfanilamide, equivalent to 1 to 20 mg per cent fluids, are also measured into test tubes. After the addition of 1 drop of 1.5 N HCl, the pH of each solution will range from 2.0 to 2.5. This degree of acidity prevents any reaction between the reagent and the nitrogenous constituents normally present in the spinal fluid. One cubic centimeter of freshly prepared 0.05 per cent sodium-beta naphthoquinone 4 sulfonate (Eastman No. 1372) is added to each tube,

TABLE I

THE RECOVERY OF SULFANILAMIDE ADDED TO CEREBROSPINAL FLUID BY THE BETA NAPHTHOQUINONE SULFONIC ACID AND MARSHALL DIAZOTIZATION METHODS

SULFANILAMIDE ADDED	SULFANILAMIDE FOUND BY THE BETA NAPHTHOQUINONE-SULFONIC ACID METHOD	ERROR	SULFANILAMIDE FOUND BY MARSHALL'S METHOD	ERROR
mg/100 cc	mg/100 cc	per cent	mg/100 cc	per cent
20.0	20.41	+2.05	20.51	+2.55
15.0	15.23	+1.53	15.46	+3.00
10.0	9.85	-1.50	9.80	-2.00
7.5	7.35	-2.00	7.58	+1.07
5.0	5.00	0.00	4.05	-1.00
2.5	2.51	+0.40	2.48	-0.50
1.0	1.05	+5.00	0.90	-1.00

then contents mixed and placed in the dark for one hour. The unknown is now read in the colorimeter against the standard which it most closely matches. The orange red color given by the reaction products shows poor proportionality, hence the standard and unknown must be of approximately the same concentration. It is generally necessary to prepare 2, 4, 7, 10, 15, and 20 mg per cent standards. After the colors have been developed any two standards may be mixed should an intermediate standard be required to secure a match with the unknown.

TABLE II

COMPARATIVE ANALYSIS OF THE CEREBROSPINAL FLUID OF PATIENTS UNDERGOING SULFANILAMIDE THERAPY

EXP NO	BETA NAPHTHOQUINONE SULFONIC ACID METHOD	MARSHALL'S METHOD	DIFFERENCE	CLINICAL DIAGNOSIS
1	15.1*	14.70*	-0.40*	Meningococcus meningitis
2	15.0	15.4	+0.4	Meningococcus meningitis
3	10.0	9.74	-0.26	Meningococcus meningitis
4	7.62	7.70	+0.08	Streptococcus viridans meningitis
5	6.96	7.4	+0.44	Streptococcus viridans meningitis
6	2.35	1.82	-0.53	Streptococcus viridans meningitis

*The values are given as mg sulfanilamide per 100 cc of cerebrospinal fluid

Measured quantities of sulfanilamide added to spinal fluid were fully and accurately recovered. Thus 1 cc of stock solution containing 2 mg of sulfanilamide was added to 9 cc of pooled spinal fluid, yielding a 20 mg per cent fluid. In a similar manner spinal fluids of various sulfanilamide content were prepared and analyzed as outlined above. In addition 3 cc quantities of each of the sulfanilamide containing spinal fluids were treated with 27 cc

of alcohol, and the corresponding filtrates subjected to analysis according to Marshall's original procedure.⁶ The data in Table I reveal the fact that excellent sulfanilamide recovery can be secured by both methods. Cerebrospinal fluids secured from patients undergoing sulfanilamide therapy were likewise subjected to analysis. The data in Table II show that both methods yield values which check closely with each other. Marshall's method is characterized by greater color intensity and better proportionality, the naphthoquinone-sulfonic acid method by greater simplicity.

SUMMARY

1. A simple method employing sodium-beta-naphthoquinone-4-sulfonate has been developed which accurately determines the sulfanilamide content of cerebrospinal fluid. Filtrate preparation is not required.

2. The values given by this method on spinal fluids secured from patients undergoing sulfanilamide therapy were found to check closely with the values given by Marshall's *diazotization method*.

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THE DETERMINATION OF PARA AMINOBENZENE SULFONAMIDE (SULFANILAMIDE) IN URINE AND BLOOD*

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THE increasingly widespread use of sulfanilamide suggested the probability that various materials might be submitted to the public health and chemical laboratory for examination and determination of sulfanilamide content. A satisfactory method for such determinations was therefore sought. This investigation was begun before the deaths were reported from the so called "Elixir of Sulfanilamide".¹

The general method of Marshall, Emelson, and Cutting² was adopted. This method involves diazotization of the amino group of sulfanilamide by nitrous acid and its subsequent coupling with dimethyl alphanaphthylamine to form a colored compound. The various concentrations of this compound may be readily determined by colorimetric comparison with standard solutions of sulfanilamide similarly treated. Several compounds other than dimethyl alphanaphthylamine however, were tried for coupling with the diazotized sulfanilamide.

The following compounds, because of their chemical structure, seemed to offer the best possibilities and the results are as follows:

(1) *Dimethylaniline*—Good gradation of orange color developed in ten to fifteen minutes. Blank lemon yellow.

(2) "*H*" Acid (1 *Naphthol-3, 6-Disulfonic Acid, 8 Amino*)—Good gradation of color running from orange to red with increasing concentration of sulfanilamide, developed in fifteen minutes. Blank yellow.

(3) *Amino "G" Acid* (2 *Naphthylamine 6, 8 Disulfonic Acid*)—Of no value. Color slow in developing. Difficult to compare.

(4) *Beta naphthylaminesulfonic Acid "F"* (2 *Naphthylamine 7 Sulfonic Acid*)—No value, golden yellow in blank as well as in standard solutions of sulfanilamide.

(5) *Amido "R" Acid* (2 *Naphthylamine, 3, 6 Disulfonic Acid*)—No value, pale yellow color only after long standing.

(6) *Metaphenylenediamine*—No value.

(7) *Diphenylamine*—Good color gradation from colorless in blank to golden yellow of increasing intensity with increasing concentration of sulfanilamide. Color best after ten to fifteen minutes. This substance (diphenylamine), on the basis of the consistent results obtained, was adopted as the standard reagent for making the determinations for sulfanilamide. A difference in concentration of 0.1 mg sulfanilamide per 100 cc of solution could be readily differentiated.

*From the Department of Public Health, San Francisco.
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The method used by Marshall, Emerson, and Cutting² will determine the sulfanilamide in any combination to which it may be changed within the human body, so long as the amino group is intact and free, or in which the amino group may be rendered free quantitatively by hydrolysis before determination. This holds true with the modified method as here presented.

The method suggested in this paper is as follows:

Reagents.—0.1 N hydrochloric acid.

0.1 per cent aqueous solution of sodium nitrite (freshly prepared).

0.5 per cent solution of diphenylamine in 95 per cent alcohol.
Standard solution of 200 mg. sulfanilamide per liter of water, from which suitable standards for comparison may be prepared.
95 per cent alcohol.

Urine.—Dilute about 1:50, to contain approximately 0.5 to 1.5 mg. sulfanilamide per 100 c.c. To 10.0 c.c. of diluted urine, add 2.0 c.c. of 0.1 N HCl, 1.0 c.c. sodium nitrite solution, and 5.0 c.c. of alcohol in turn with shaking. After three to five minutes, add 1.0 c.c. of the diphenylamine solution. Compare with standard sulfanilamide solutions similarly treated. The comparison is best made about fifteen minutes after adding the diphenylamine.

Marshall, Emerson, and Cutting² have shown that sulfanilamide is partly excreted in a conjugated form (largely para-acetylaminobenzene sulfonamide) which may be determined only after hydrolysis to free the amino group. This hydrolysis is accomplished by heating for thirty minutes in boiling water equal volumes of urine and normal hydrochloric acid. The solution is then cooled, one drop of phenolphthalein solution added, and neutralized with 2 normal sodium hydroxide. The resultant solution is then diluted and treated as above.

The method of Marshall and his associates, as here modified, was found to give consistent results on human urine following administration of sulfanilamide. As indicated by determinations before and after hydrolysis, as much as 40 per cent of the sulfanilamide may be excreted in the conjugated form.

Blood.—The method of Marshall and his coworkers was used for determining free sulfanilamide in the blood, except for the use of diphenylamine to produce color. One volume of blood is run slowly with shaking into 9 volumes alcohol, stoppered, and let stand fifteen minutes. The mixture is filtered and 10.0 c.c. of filtrate measured into a small flask. Five cubic centimeters of water, 2.0 c.c. of 0.1 N HCl, and 1.0 c.c. of sodium nitrite solution are added with shaking. After three to five minutes, 1.0 c.c. of the diphenylamine solution is added. The colored solution is turbid, but may be filtered after about five minutes, and the filtrate used for colorimetric comparison. Standards are prepared by adding 1.0 c.c. of sulfanilamide solution of suitable strength to 9.0 c.c. of alcohol and treating this solution in the same way as the blood filtrate. The color is best about fifteen minutes after addition of the diphenylamine.

For the determination of hydrolyzable conjugated compounds of sulfanilamide in the blood, the following method has yielded the best experimental results:

To 100 cc of the blood filtrate as prepared above, add 50 cc of 0.1 N HCl, heat in boiling water for thirty minutes with reflux condenser. The solution is cooled and treated as previously described, omitting the addition of 50 cc of water and the hydrochloric acid. Standards are prepared as before, except that no water is added, and 5 cc of 0.1 N HCl are added in place of 2 cc. However, use of the standards as prepared for determination of the free sulfanilamide in blood will apparently produce an error of not more than 10 per cent.

In order to determine whether or not some of the commonly used drugs which contain amino groups might produce color which could be mistaken for an indication of the presence of sulfanilamide in urine, urine was collected after ingestion of acetanilide and after acetphenetidin. Neither sample produced color under the conditions of the determination, either before or after hydrolysis with hydrochloric acid.

SUMMARY

1 The method presented for hydrolyzing conjugated compounds of sulfanilamide in the blood is perhaps of more value than is the change from dimethyl alphanaphthylamine to diphenylamine in the determination.

2 It is found that as much as 40 per cent of the sulfanilamide excreted in the urine may be in a conjugated form.

3 Two common drugs, acetanilide and acetphenetidin, are shown not to interfere with determination of sulfanilamide in urine.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

WEIL'S DISEASE, Report of Seven Cases, Gaines, A. R., and Johnson, R. P. Arch. Int. Med. 60: 817, 1937.

The diagnosing of Weil's disease in seven cases, six of them being noted within a period of six months, indicates that this condition may not be rare.

Because of the fact that five showed an atypical course, according to the standard textbook picture, the authors believe that this disease should be considered more often when there is unexplained jaundice.

The source and mode of infection in these cases have not been determined. This is true not only for this series but also for the previously reported cases in North America, except for one instance of accidental laboratory infection.

TRANSFUSION, Reaction Following Intra Group, Culbertson, C. G., and Ratcliffe, A. B. Am. J. M. Sc. 192: 456, 1937.

The relation of anomalous agglutinins to transfusion has been an unsettled question. It might be questioned that the agglutinin was responsible for the reaction in this case. However, the facts may be summarized as follows: a Group O patient was given 500 c.c. of Group O blood; the cells of this blood were susceptible to agglutination by an anomalous agglutinin of low titer existing in the serum of the recipient. The recipient exhibited the typical symptoms of a delayed transfusion reaction, followed a typical course, and eventually recovered. A second patient, exhibiting a similar anomalous agglutinin, was given 550 c.c. of blood compatible with her intragroup agglutinin, and no sign of reaction was observed. Until the contrary is shown, the authors shall continue to regard incompatibility resulting from anomalous agglutinins as a definite contraindication to transfusion with such a blood.

In each of the 2 cases the low-titer intragroup agglutinin was unobserved in the routine compatibility tests by the hanging-drop method, although it was readily demonstrated by the centrifuge test method.

They suggest the use of the centrifuge test method given below as an aid in avoiding transfusion reactions of the type mentioned above.

Technique for the Centrifuge Test Method:

1. Place 3 drops of serum in a small test tube (14 mm. diameter).
2. Add 1 drop of cell suspension (3 drops of blood from a 20-gauge needle in 4 c.c. of a physiologic saline solution containing 0.2 per cent sodium citrate).
3. Shake to mix. (A control of saline and cells is optional after a little experience.)
4. Incubate five minutes at 37° C.
5. Centrifuge three minutes at high speed.
6. Note any evidence of hemolysis.
7. Shake the tubes to resuspend the cells while observing them with a concave mirror or hand lens. Note any evidence of clumping.
8. Pour the fluid onto a glass slide and examine microscopically for agglutination.

BLOOD GROUP, Relation of, to Blood Diseases and Neoplasms, MacLeod, K. I. E. Brit. M. J., p. 745, October 16, 1937.

Before any conclusions can be formed as to the relation of the blood group of the individual to a particular disease, the facts and figures from a very large selection of cases must be examined.

This is only feasible if the observer or observers have the necessary time to spend on the laborious testing of these cases, which must be very accurately diagnosed to be of any statistical value

It is more feasible, however, for a multitude of observers to note their findings regarding this relation of the blood group to the cases of any diseases at their disposal or to those of a particular disease in which they are interested, and having collected their notes, to submit them to a common group of statisticians who could file the material. When sufficient data had been collected, conclusions of probable interest and possible value might be derived from them

For contrast as well as control purposes, cases from the two classes of diseases chosen for this article were blood grouped. Some interesting figures were noted

Whereas for general purposes the total percentages of blood groups of the cases of neoplasms grouped were roughly within normal limits, it was noted, on the other hand, that there was a strikingly high incidence of blood diseases in the blood groups B and AB. It was also noted that many of the severest cases of the blood diseases fell into those latter blood groups

Several cases in which autogglutination was present were grouped, and it was interesting to note one or two points about this phenomenon

One would have liked to have more time to spend and a larger number of cases at one's disposal, in order that some definite ruling as to the ultimate direction of these figures could be established

MILK, Phosphatase Test for Detection of Underpasteurized Milk, Geiger, J C, and Davis, C J A M A 109 1363, 1937

The experience of the San Francisco Department of Public Health with the phosphatase test as advocated by Kay and Graham would indicate that adequately pasteurized milk will invariably give a negative test

This test, therefore, offers reliable laboratory evidence to confirm proper pasteurization of milk and cream of market supplies

The test briefly is as follows

1 To 10 cc of the buffer substrate solution (one tablet containing disodium phenyl phosphite and sodium barbitalone is dissolved in 50 cc of water) contained in a 25 cc. stoppered test tube, add 0.5 cc of the milk and mix thoroughly

2 Add two drops of chloroform stopper the tube, and incubate at from 37° to 38° C for one hour

3 At the end of this time, add 4.5 cc of the diluted Folin and Ciocalteu reagent (1 volume of reagent and 2 volumes of water), mix, allow to stand for three minutes and filter

4 To 10 cc of the filtrate add 2 cc of the sodium carbonate solution (14 per cent anhydrous sodium carbonate), mix thoroughly, and place in boiling water for five minutes and again filter

5 Compare the color of the filtrate in a 13 mm tube with the Lovibond standard 2.3 blue glass in the "hmitaster"

If the color of the filtrate exceeds that of the standard glass, it may be safely assumed that the milk has been improperly pasteurized, that is, the temperature has been too low or the time of heating too short

BISMUTH Rapid Clinical Method for Estimation of, in Urine, Hanzlik, P J, Lehman, A J, Richardson, A P and Van Winkle, W, Jr Arch Dermat & Syph 36 725, 1937

The method makes use of well known principles of direct oxidation by potassium permanganate, sulfuric acid and heat decolorization with oxalic acid to the clearance of water, and of a reducing agent to prevent oxidation of sodium iodide for reaction with bismuth in a strongly acid solution. The iodide reacts with bismuth to form sodium iodobismuthate, a colored ion which permits direct colorimetric estimation by matching with permanent

color standards on a paper scale. Only about ten minutes is required for making an estimation. The equipment is as simple as that used for other laboratory work in clinics or physicians' offices, and is readily procurable. It consists of a small iron support, a clamp, a pyrex test tube (3 by 20 cm.), and a microburner. All reagents, except concentrated sulfuric acid, are obtainable in tablet form. Undoubtedly the worst reagent to handle is concentrated sulfuric acid, but nothing has been found that will take its place. A large test tube is necessary to accommodate foaming, which is readily controlled by playing the flame of the microburner up and down the sides of the test tube. With a little practice one can easily manipulate the test tube without loss of its contents. However, a loss does not affect the accuracy of the estimation, which depends on matching concentrations.

The paper color scale was prepared by Mr. George H. Needham, of San Francisco, an expert water-color artist and microchemist, who matched standard solutions of bismuth and the same reagents (obtainable from the James H. Barry Co., 170 Van Ness Avenue, South, San Francisco). Six color standards, indicating quantities of bismuth ranging from 0.25 to 1.2 mg. per hundred cubic centimeters, are represented on the paper scale. Intermediate quantities of bismuth may be estimated by interpolation. It would be possible, of course, to use more color standards for estimating intermediate quantities with a greater range of accuracy, but this would make the scale unwieldy. A legend below the color standards gives the following information: milligrams of bismuth per hundred cubic centimeters, daily output of bismuth in 1,200 c.c. of urine (when the daily volume is known an estimation can be made accordingly), and the details of the procedure for testing urine.

The estimation of bismuth is conveniently carried out in six steps as follows:

1. Put 10 c.c. of urine into a long test tube (3 by 20 cm.).
2. Add 1 tablet (0.4 gm.) of potassium permanganate and 2 c.c. concentrated sulfuric acid. Heating will produce foaming.
3. Boil gently over a microburner for about two minutes.
4. Add 1 tablet (0.4 gm.) of oxalic acid. Decolorization will take place, and the solution should be allowed to cool.
5. Add 1 tablet (from 0.01 to 0.04 gm.) of sodium sulfite and sodium sulfate and 1 tablet (0.05 gm.) of sodium iodide. The fluid will become yellowish green if bismuth is present.
6. Match with the color scale, holding the test tube against the white margin above the standards.

If for an occasional specimen complete oxidation does not take place, indicated by the presence of some tint, the procedure from the second step on should be repeated.

The final oxidized solution must be clear as water for the proper matching of colors.

ARTHRITIS, Gonococcic, Pathogenesis, Mechanism of Recovery and Treatment, Keefe, C. S., and Spink, W. W. J. A. M. A. 109: 1448, 1937.

A study of 140 cases of gonococcic arthritis leads to the following statements:

Gonorrhea is a frequent cause of acute polyarthritis, and, while any joint may be affected, gonococcic arthritis is most often seen in the knees, wrists, and ankles.

Associated lesions, such as tenosynovitis, bilateral metastatic catarrhal conjunctivitis, iridocyclitis, and keratoderma blennorrhagicum, are helpful clinical aids in the diagnosis.

The pathologic lesions in the joints begin in the periarticular tissues and synovial membrane, and involvement of the cartilage is secondary. Destruction of cartilage is most conspicuous in the wrist, hip, and finger joints. Bony ankylosis is rare except in the wrist joints. Fibrous ankylosis is more common.

Examination of the synovial fluid is helpful in diagnosis. Organisms were isolated in 26 per cent of the cases. The fluid had all the characteristics of an exudate. The average total cell count was higher for the infected fluid than for the sterile fluid.

Gonococcus complement fixation and bacteriolytic antibodies diffuse into the synovial cavities.

The antibody content of synovial fluid is the same or slightly less than that of the blood when the fluid is sterile. When it is infected, antibodies are not demonstrated.

The synovial fluid contains antitryptic substances which probably protect the cartilage from destruction by the tryptic like ferments of the leucocytes

The gonococcus complement fixation test on the blood gave a positive reaction in 86 per cent of the cases. The percentage of positive reactions was somewhat lower for tests on the synovial fluid, especially when the fluid was infected

The mucin in synovial fluid interferes with the destruction of organisms when the antibody titer is low. When the antibody titer rises there is no apparent depression of the activity of the fluid

The blood plasma of patients with gonococcal infections is actively bactericidal for the homologous strain of infecting organism. The bactericidal activity increases during the course of the disease

There is suggestive evidence that recovery from gonococcal arthritis is associated with the development of increased resistance to the invading organism

There are various types of treatment. There is evidence that sulfanilamide is bactericidal for the gonococcus when it is added to blood serum. In two patients with infected synovial fluid the gonococci disappeared from the fluid within two days after this drug was administered. This type of treatment deserves further study and investigation

PNEUMONIA. Pneumococcal, Community Provision for Serum Treatment of, Cecil B. L. Bullock, J. G. M. Chickering, H. T. and Corwin, E. H. L. J. A. M. A. 109 1323 1937

During the next few years departments of health should engage in a vigorous campaign against pneumonia. Special divisions of pneumonia service should be established under the guidance of properly qualified physicians

Through the regular medical channels, physicians should be made cognizant of the fact that serum is life-saving in certain types of pneumonia and that the particular type of pneumococcal infection from which the patient may be suffering should be determined at the earliest possible moment. Free facilities for the rapid determination of the type of infection should be made available in each community at all times, day and night

Because of the communicable nature of the pneumonias it is highly desirable that pneumonia patients in hospitals be segregated in cubicles and that a complete aseptic technique be followed

Pneumonia patients should be considered in the same urgent category with emergency surgical cases. Certain physicians on the attending staff should be made responsible for the treatment of these patients and should be on call day and night as is the custom in the surgical services

In connection with the divisions of pneumonia service of health departments, a clinical consultation service should be established to aid physicians in the administration of serum therapy and in the taking of specimens of blood and sputum for bacteriologic study

In all instances of death from pneumonia, physicians should be requested to report the precise nature of the invading organism

Concentrated serum for the prevailing types of pneumonia should be made available without cost to physicians requesting it provided the type of pneumonia has been ascertained prior to the request for serum

Control work of pneumonia and the production of therapeutic serums should not be allowed to interfere with the fundamental research activities of the laboratories

The results obtained in New York and Massachusetts justify the appropriation of adequate funds to health departments for pneumonia control work and continued research

HORMONES, Serologic Antibodies Against, Bauer, J. J. A. M. A. 109 1442, 1937

A prolonged treatment of rabbits with injections of thyroxine leads to a resistance of these animals against thyroxine and to the appearance of serologic antibodies detectable by the complement fixation reaction

color standards on a paper scale. Only about ten minutes is required for making an estimation. The equipment is as simple as that used for other laboratory work in clinics or physicians' offices, and is readily procurable. It consists of a small iron support, a clamp, a pyrex test tube (3 by 20 cm.), and a microburner. All reagents, except concentrated sulfuric acid, are obtainable in tablet form. Undoubtedly the worst reagent to handle is concentrated sulfuric acid, but nothing has been found that will take its place. A large test tube is necessary to accommodate foaming, which is readily controlled by playing the flame of the microburner up and down the sides of the test tube. With a little practice one can easily manipulate the test tube without loss of its contents. However, a loss does not affect the accuracy of the estimation, which depends on matching concentrations.

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Gonococcus complement fixation and bacteriolytic antibodies diffuse into the synovial cavities.

The antibody content of synovial fluid is the same or slightly less than that of the blood when the fluid is sterile. When it is infected, antibodies are not demonstrated.

test for syphilis, to prevent the transmission of the disease. The Kline exclusion slide test is an excellent method for this purpose, even in emergency cases, because of the rapidity, simplicity, and ease of its performance, and its high degree of sensitivity.

BLOOD SEDIMENTATION In Pulmonary Tuberculosis, Volk, R. Am Rev Tuberc 36: 567, 1937

Study of the results obtained on 1,000 sedimentation determinations shows a definite parallelism between clinical findings and sedimentation rates. Patients having clinical signs of active disease present the greatest percentage of "retic curves." There was agreement to the extent of 85 to 99 per cent which is as close a check as anyone can hope for in a nonspecific procedure.

There seems to be definite evidence that red cell sedimentation can be a valuable adjunct to other routine procedures in the clinical study of tuberculosis. It has been found of considerable value in pneumothorax and thorico-plasty cases, where x-ray films may give us no more information than that a certain amount of collapse of the lung exists.

A repeatedly elevated sedimentation rate, with no clinical signs of activity of tuberculous disease, requires a more careful study of the x-ray films, more thorough examinations of sputa, and, if these continue to be negative, the patient should be investigated for other causes of an increased sedimentation rate.

The red cell sedimentation should not be depended upon exclusively, because there are other factors to alter the test in individual cases, such as severe anemia, pregnancy, and intercurrent infections, but the test has shown itself of sufficient value to be included as a routine procedure in tuberculosis sanatoria.

TUBERCLE BACILLI, Comparative Study of Various Methods of Cultivation From the Blood, Galton, M. M. Am J Hyg 26 299, 1937

From this study on the cultivation of *Mycobacterium tuberculosis* from blood the following conclusions may be drawn:

- 1 The use of untreated blood and water hemolyzed blood sediment yielded a fair and approximately equal percentage of cultures. The acetic acid method was entirely unsatisfactory in the hands of the author.

- 2 With all types of the blood samples, Petragraní medium and extract blood agar were the best of the ten media employed, both with respect to the number of successful cultures and the earliest appearance of growth.

- 3 All egg media, except that of Petragraní, were inferior to extract blood agar.

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THOSE familiar with previous editions of this book—and they must be legion for this is a book to gladden the soul of the practitioner and, most especially, the young practitioner—will recall that it deals almost entirely with the homely, everyday conditions, about which the physician is apt to be most frequently consulted.

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*Minor Maladies and Their Treatment. By Leonard Williams, M.D. Cloth, ed 7 420 pages \$3.75 William Wood & Co., Baltimore, Md.

†Muir's Bacteriological Atlas. By C. D. Van Reogen, M.D. Harley Research Fellow and Lecturer in Bacteriology, University of Edinburgh. Cloth, ed 2, 90 pages, 83 colored plates, \$5.25 William Wood & Co., Baltimore, Md.

‡Modern Treatment in General Practice, Vol. III. Edited by Cecil P. G. Wakeley, D.Sc., Fellow of King's College, London, Senior Surgeon, King's College Hospital. Editor of "The Medical Press and Circular." Cloth, 436 pages, 54 figures \$4.00 William Wood & Co., Baltimore, Md.

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IT IS hardly conceivable that there could be any one engaged in the study of animal or plant histology unfamiliar with Lee's Vade Mecum, for it has been an invaluable standard reference text for 10¹ these many years.

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As in the previous edition, each chapter is followed by a concise summary and by a list of references.

*The Microtomist's Vade Mecum (Bates Lee). A Handbook of the Methods and Animal and Plant Microscopic Anatomy. Edited by J. Bronte Gatenby, Professor of Zoology and Comparative Anatomy, Trinity College, Dublin, and Theophilus S. Painter, Professor of Zoology, Texas University. Cloth ed 10 "84 pages \$3.00 P. Blakiston's Son & Co. Inc. Philadelphia, Pa.

†Practical Physiological Chemistry. By Philip P. Hawk, M.S. Ph.D. and Olaf Bergheim, M.S. Ph.D. in collaboration with Bernard L. Oser, Ph.D. and Arthur G. Cole, M.D. Cloth ed 11 "86 pages 275 figures 1 colored plate \$8.00 P. Blakiston's Son & Co. Inc. Philadelphia, Pa.

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The text is clear-cut, of easy style, and embodies a wealth of valuable information based, not only upon research, but also upon extensive clinical experience. In addition to reference lists appended to separate chapters there are an extensive author index and a comprehensive general index. The format is pleasing, and the illustrations excellently reproduced.

Both author and publisher may be congratulated upon an outstanding book destined, without doubt, to become a standard reference upon this subject.

A Method of Anatomy†

THIS book should prove a boon to the student, to the physician, surgeon, pathologist, and, indeed, to all concerned with human anatomy. As the author comments in his preface, one may learn anatomy by laboriously memorizing facts, or by correlation of facts through study of their mutual relationships. It is from the latter angle that this book has been written—and well written, indeed.

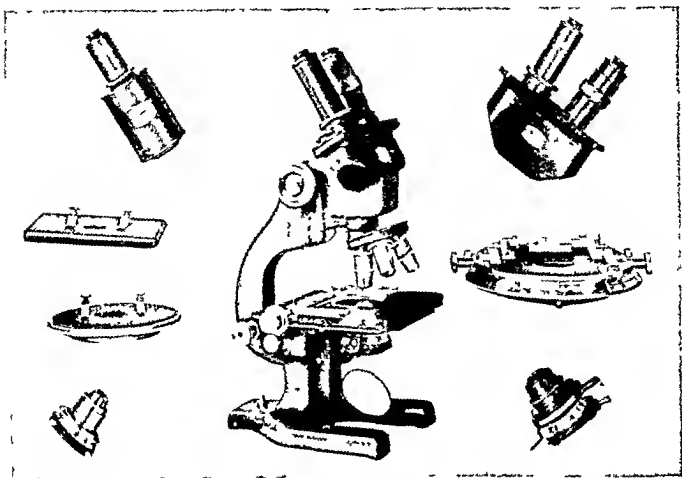
A feature of particular value is the illustrations—clear-cut, simple, striking in their ability to convey a definite idea.

While diagrammatic, they are nevertheless accurate in relationships and based upon accurate measurements and careful dissections.

Professor Grant has written a book which seems destined to take its place as a classic on this subject, and one which can be highly recommended without reserve.

*The Therapeutic Problem in Bowel Obstructions. By Owen H. Wangensteen, M.D., Professor of Surgery, University of Minnesota. Cloth, 360 pages, 90 figures. \$6.00. Charles C. Thomas, Springfield, Ill.

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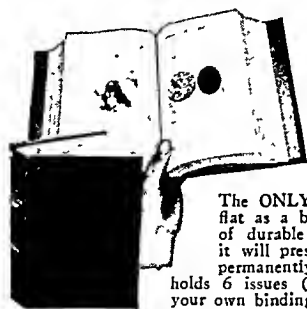
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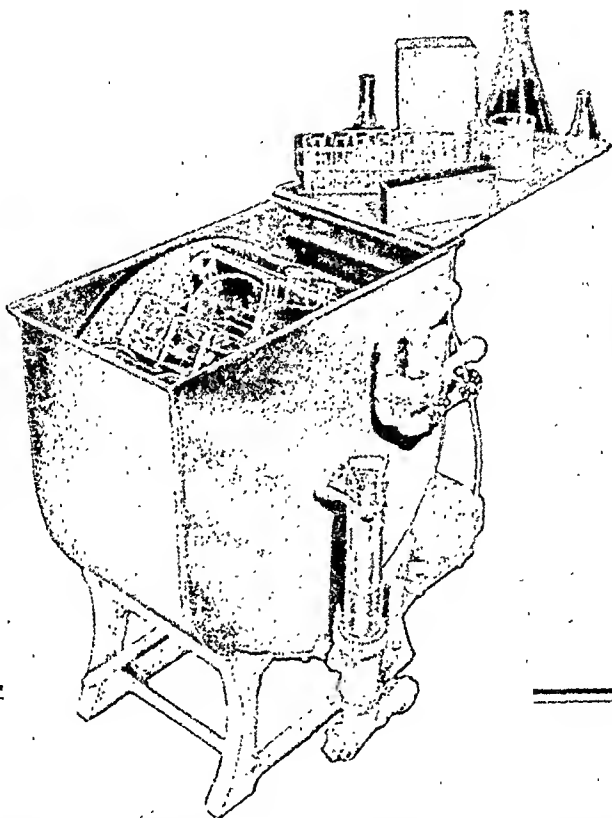
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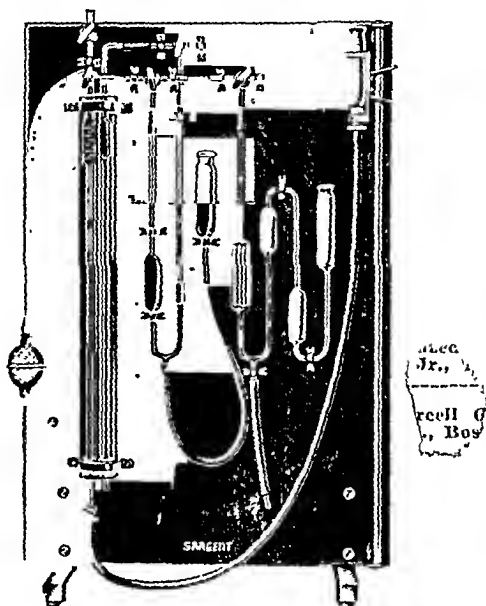
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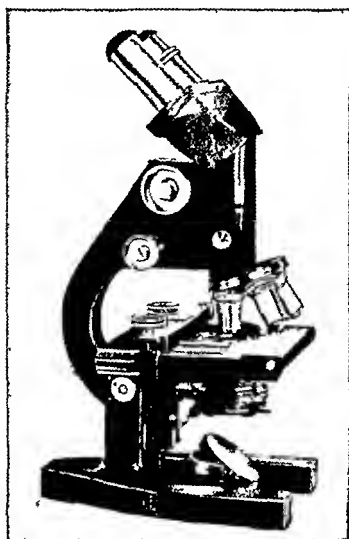


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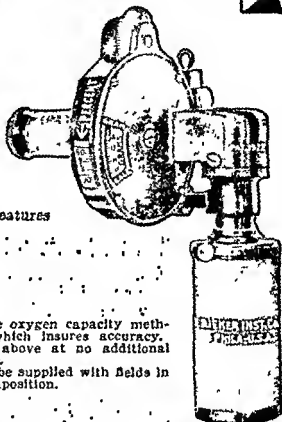
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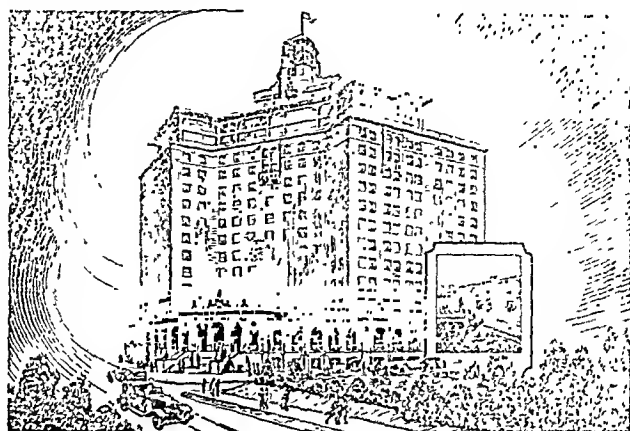
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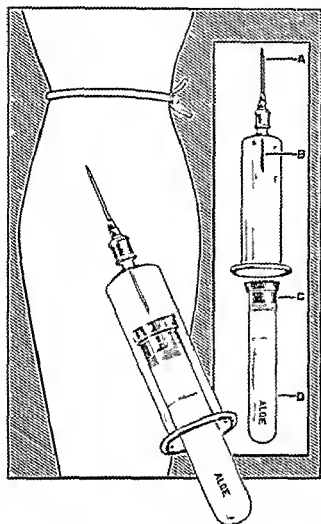
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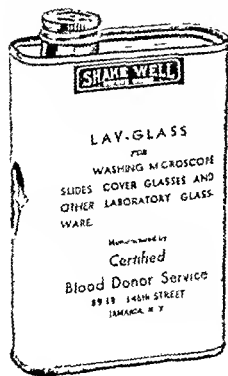
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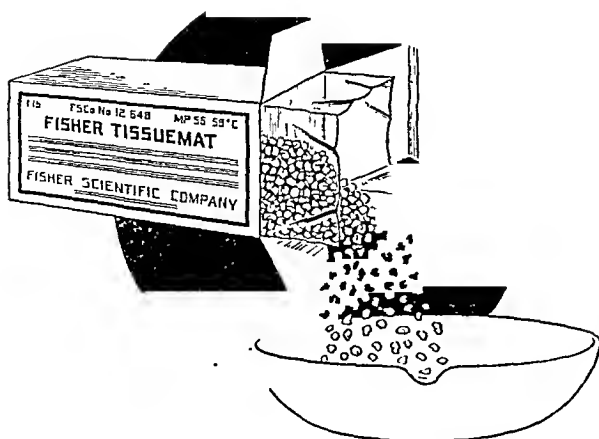
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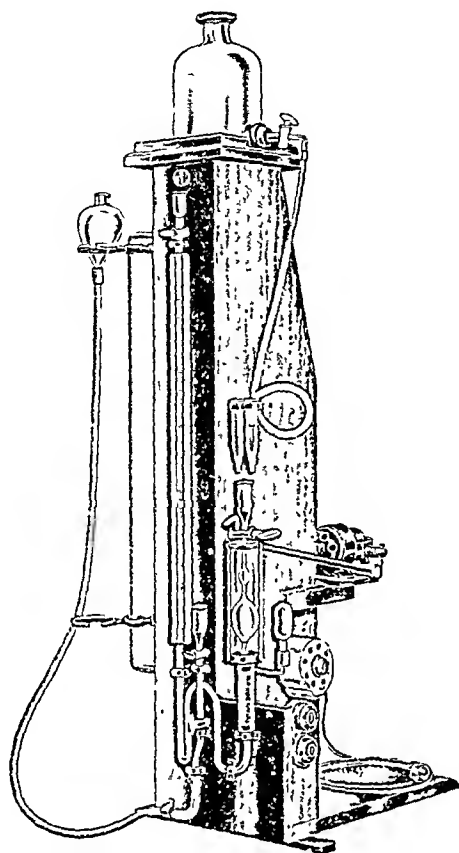
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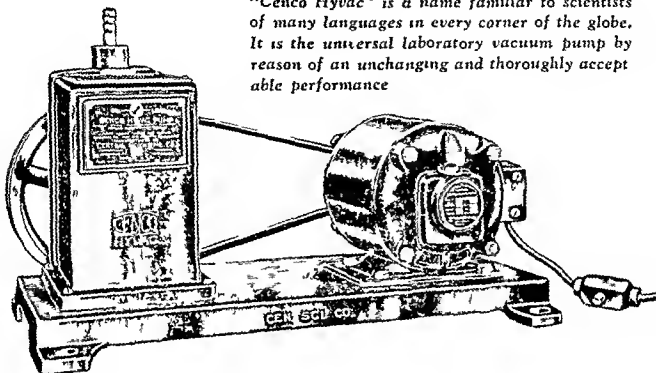
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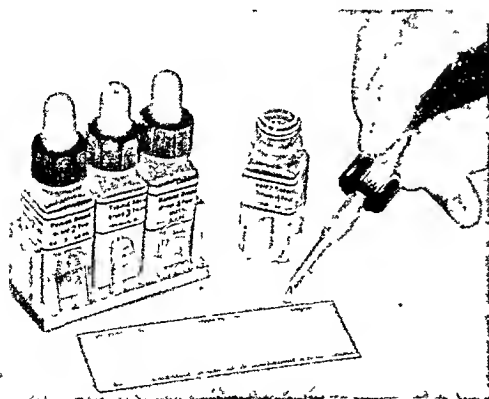
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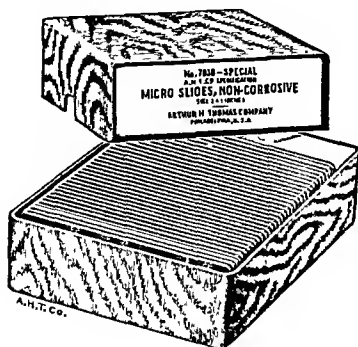


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The Journal of Laboratory and Clinical Medicine

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No 7

CLINICAL AND EXPERIMENTAL

INADEQUACY OF CONJUNCTIVAL SMEARS IN THE DIAGNOSIS OF SLIGHT VITAMIN A DEFICIENCY IN ADULTS*

JOHN B. YOUNG, M.D., F.A.C.P., MARVIN B. CORLETT, M.D., MILDRED G.
(ORLETT), B.S. AND HELEN FRANK, B.A., NASHVILLE, TENN.

THE classical signs and symptoms of vitamin A lack are, in truth, manifestations of the relatively rare cases of severe deficiency and as such are of little value in detecting the milder or earlier cases of this disease. Most, if not all, of the early ocular symptoms (photophobia, asthenopia, burning of the eyes, etc.) are so unspecific that although they may serve to suggest the deficiency, as may a scrutiny of the diet, they are insufficient evidence on which to base a diagnosis. If, as now seems probable, cases of mild deficiency are frequent and often escape recognition, there is a need for clinical methods of diagnosing them.

The methods which have recently been used for the detection of slight deficiency of vitamin A are (1) the determination of dark adaptation, using various types of photometers, (2) the examination of smears made from scrapings of the conjunctivae and other epithelial surfaces, and (3) the presence of changes in the skin. Changes in the skin, although they apparently may occur unassociated with xerophthalmia or outspoken night blindness, do occur with those more serious symptoms and when extensive are themselves manifestations of severe deficiency.¹ They are, moreover, inconstant and in children infrequent. Therefore, although when they occur they may be diagnostic of vitamin A deficiency, even in the absence of the severe eye symptoms, and may occur as evidence of relatively mild deficiency, they are not suitable as a reasonably constant diagnostic sign of mild vitamin A deficiency.

*From the Department of Medicine, Vanderbilt University School of Medicine, Nashville.
This study was aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

Received for publication Sept. 9, 1937.

Such studies as those of Jeans and Zentmire,² Frandsen,³ Jeghers,⁴ and others suggest that the presence of a slight hemeralopia or lowered dark adaptation constitutes the earliest and most reliable evidence of slight vitamin A deficiency. These studies have not, however, been fully completed nor standards finally established, and the test requires the use of rather expensive apparatus. The method is, furthermore, difficult or impossible to use in examining small children or uncooperative adults.

Changes in the cells of the various epithelial surfaces occur relatively early in vitamin A deficiency, according to the studies of Blackfan and Wolbach⁵ and others. Among the various places where these changes occur are the eye, nose, mouth, bronchi, vagina, pelvis of the kidney and ureters, but for various reasons, the epithelium of the conjunctiva offers the most suitable clinical site from which to obtain material for the study of these changes.

It is clear that if changes in the epithelium observed in smears from scrapings of the conjunctivae are reliable evidence of early or slight vitamin A deficiency, they provide a quick and simple method for diagnosing this disease. Sweet and K'ang⁶ believe that they offer reliable evidence of slight and early deficiency, but fail to define fully the degree of the deficiency. Several of their patients had rather far-advanced changes in the eyes. We have found cornification of the epithelium of the conjunctivae in patients suspected of vitamin A deficiency and have observed improvement following the administration of cod liver oil. However, as far as we are aware, there has been no report of a study of smears from normal adults, and no standards have been established by which one may judge a deviation from the normal or the significance of varying degrees of cornification of these epithelial cells. In other words, no one has determined what kind and degree of changes observable in the conjunctival smears, if any, warrant a diagnosis of vitamin A deficiency before the appearance of rather gross changes in the eye. It was the purpose of this study to determine the character of the cells obtained from scrapings of the bulbar conjunctivae in normal subjects and those suspected of an inadequate intake of vitamin A, and to attempt to set up such standards. Because the optimal requirement of vitamin A is unknown and because we had learned from previous experience that subjects considered normal might be suffering from slight vitamin deficiency, the study included observations on the effect of added vitamin A.

METHODS

The normal subjects selected for this study were presumably healthy adults; staff, students, technicians, and nurses. Their diets were of a kind usually considered to be adequate in vitamin A, and all were believed to be free from local or general disease. The patient group consisted of persons suffering from minor illness not directly affecting the eyes, whose diets were suspected of being inadequate in vitamin A. Thus, patients with other evidence of mild vitamin A deficiency were included. Smears were made from light scrapings of the bulbar conjunctiva of each eye, the eyelids being held open for a minute or two before-hand to cause, if possible, a slight drying of the conjunctivae. No local anesthetic was used, and the procedure was painless and only occasionally slightly

distressing. The scrapings were made with a small stainless steel spatula. Two or more smears were made at varying intervals in most cases and from all who were given vitamin A. In the latter group the last preliminary smear was made immediately before the administration of vitamin A was started, the previous smear having been made within three to four weeks earlier in all but two subjects. Each of the subjects receiving vitamin A was given 25,000 units daily by mouth for thirty days in the form of halibut liver oil*. At the end of the thirty days, smears were again made and compared with those obtained before giving the vitamin A.

The smears were stained with hematoxylin for an hour, washed, deeply blued by passing through ammonia vapor, again washed, and counterstained faintly with eosin to a degree sufficient to stain the cornified cells, but not the others. The smears were then washed again, dried, and examined. All the cells were counted except when large clumps were present.

RESULTS

It was evident at once that varying degrees of cornification occur in the cells from the conjunctivae of normal subjects. All stages from the earliest changes in staining and slight alterations in the nuclei to complete cornification were observed. It became necessary, therefore, to establish criteria for the classification of the cells. Attempts were made to establish several grades of cornification, but in consideration of the difficulty in interpreting fine shades of change, the frequent minor variations in staining technique and the need for a sharp, easily determined criterion for clinical use it was deemed best to make a simple division into nucleated and nonnucleated cells. The former were considered for the present purposes, normal cells; the latter cornified. In addition to the loss of nuclei the abnormal cells are often larger than normal, irregular in outline, and stained pink or not at all. It is believed that for practical purposes this classification reflects changes from the normal as effectively as a more elaborate system.

In some smears the number of cells obtained is very small so that the results are not significant. For this reason we believe that there should be at least thirty-five cells in the smears from the two eyes to make the examination satisfactory. In two subjects there were only twenty-five cells in the smears from the two eyes. The findings are shown in the accompanying tables and chart.

In fifty examinations in twenty-five normal subjects before they received added vitamin A (Table I), the average per cent of cornified cells in the smears from the two eyes was 58.8 per cent, in the separate eyes (100 smears) 60.4 per cent. However, the range was enormously great, the minimum percentage of cornified cells in the two eyes being 4 (H E), the maximum 97 (E D) per cent, the minimum in either eye 3 (H E), and the maximum 100 per cent (E D). The median for the two eyes was 62, for either eye 64 per cent. More importantly, the distribution was wide as shown in Chart I.

When smears from the two eyes, taken at the same time, were compared, a similar wide variation was found. The maximum difference was found in

*425 units of vitamin D daily were included.

TABLE I

EPITHELIAL CELLS IN SMEARS FROM THE CONJUNCTIVAE OF 25 NORMAL SUBJECTS BEFORE AND AFTER RECEIVING SUPPLEMENTARY VITAMIN A

SUBJECT	DATE	RIGHT EYE						LEFT EYE						COMBINED EYES					
		TOTAL CELLS	CORNIFIED		NORMAL			TOTAL CELLS	CORNIFIED		NORMAL			TOTAL CELLS	CORNIFIED		NORMAL		
		NO.	NO.	%	NO.	%		NO.	NO.	%	NO.	%		NO.	NO.	%	NO.	%	
1. R. W.	9/ 9/36	21	17	81	4	19	43	27	62	16	33	64	44	68	20	32			
	10/ 5/36	28	26	94	2	6	42	7	16	35	84	70	33	47	37	53			
	11/10/36*	29	24	82	5	18	106	25	23	81	77	135	49	36	86	64			
2. J. Y.†	8/31/36	18	11	65	7	35	21	16	76	5	24	39	27	70	12	30			
	9/26/36	91	41	45	50	55	38	17	44	21	56	129	58	45	71	55			
	10/24/36	64	16	25	48	75	94	24	25	70	75	158	40	25	118	75			
3. M. G. C.	8/31/36	22	18	81	4	19	123	91	74	33	26	145	109	75	36	25			
	9/28/36	25	17	68	8	32	7	5	71	2	29	32	22	67	10	33			
	10/28/36	115	23	20	92	80	104	37	35	67	65	219	60	27	159	73			
4. H. F.	9/21/36	62	21	33	44	67	193	57	29	136	71	255	78	30	177	70			
	9/28/36	55	21	38	34	62	172	45	26	127	74	227	66	29	161	71			
	10/28/36	173	38	21	135	79	251	62	24	189	76	424	100	23	324	77			
5. F. W.	6/16/36	16	14	87	2	13	17	11	65	6	35	33	25	75	8	25			
	8/25/36	28	15	53	13	47	11	5	45	6	55	39	20	51	19	49			
	12/ 1/36	59	8	13	51	87	121	20	16	101	84	180	28	15	152	83			
6. M. B. C.	8/26/36	57	33	58	24	42	42	27	64	15	36	99	60	60	39	40			
	9/28/36	24	15	62	9	38	82	34	41	48	59	106	49	40	57	54			
	10/28/36	85	24	28	61	72	31	26	83	5	17	116	50	42	66	57			
7. F. S.†	8/28/36	106	42	39	64	61	90	41	45	49	55	196	83	42	113	58			
	9/30/36	20	17	85	3	15	101	38	37	63	63	121	55	45	66	53			
	10/27/36	37	17	46	20	54	34	32	94	2	6	71	49	69	22	31			
8. A. S. M.†	8/31/36	36	27	75	9	25	32	19	60	13	40	68	46	67	22	33			
	9/28/36	38	27	71	11	29	123	12	9	111	91	161	39	24	122	76			
	10/28/36	41	20	48	21	52	587	62	10	525	90	628	82	13	546	87			
9. H. S.	8/31/36	52	34	64	18	36	13	6	46	7	54	65	40	61	25	39			
	9/29/36	52	31	61	21	39	52	48	92	4	8	104	79	76	25	24			
	10/29/36	40	36	65	14	35	49	20	41	29	59	89	46	51	43	49			
10. I. D.	9/16/36	25	19	76	6	24	20	13	65	7	35	45	32	71	13	29			
	10/ 7/36	15	13	86	2	14	47	43	92	4	8	62	56	90	6	10			
	11/ 7/36	15	15	100	0	0	21	11	52	10	48	36	26	72	10	28			
11. H. K.	9/16/36	27	19	70	8	30	61	49	80	12	20	88	68	77	20	23			
	9/29/36	34	8	23	26	77	28	8	28	20	72	62	16	25	46	75			
	10/29/36	39	30	77	9	23	212	7	3	205	97	251	37	15	214	85			
12. A. B.†	7/15/36	32	23	71	9	29	34	23	67	11	33	66	46	70	20	30			
	10/ 2/36	33	30	90	3	10	48	26	54	22	46	81	56	69	25	31			
	11/ 2/36	32	28	87	4	13	38	12	31	26	69	70	40	57	30	43			
13. P. Z.	8/26/36	74	42	56	32	44	57	36	63	21	37	131	78	59	53	41			
	9/30/36	38	24	63	14	37	19	17	90	2	10	57	41	71	16	29			
	11/ 3/36	40	25	62	15	38	55	24	43	31	57	95	49	51	46	49			
14. D. O.	9/16/36	154	91	59	63	41	23	15	65	8	35	177	106	60	71	40			
	9/29/36	34	27	79	7	21	29	24	82	5	18	63	51	81	12	19			
	10/29/36	67	36	53	31	47	180	55	30	125	70	247	91	36	156	64			
15. R. K.	8/12/36	12	6	50	6	50	7	12	70	5	30	29	18	62	11	38			
	9/29/36	40	38	95	2	5	156	58	37	98	63	196	96	49	100	51			
	10/30/36	95	80	84	15	16	550	53	11	497	89	645	133	20	512	80			
16. E. J.†	9/ 8/36	32	11	34	21	66	25	7	28	18	72	57	18	31	39	69			
	10/ 6/36	24	13	54	11	46	46	31	67	15	33	70	44	63	26	37			
	11/ 6/36	253	66	26	187	74	146	18	12	128	88	399	84	21	315	79			

*In each case, the first two readings are before administration of vitamin A; the third after.

†Subject later showed evidence of mild vitamin A deficiency by photometric test.

TABLE I- CONT'D

SUBJECT	DATE	RIGHT EYE						LEFT EYE						COMBINED EYES					
		TOTAL CELLS		CORNI FIED		NORMAL		TOTAL CELLS		CORNI FIED		NORMAL		TOTAL CELLS		CORNI FIED		NORMAL	
		NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
17 L D †	9/15/36	7	21	56	16	44	7	75	46	41	54	113	56	49	37	51			
	10/ 6/36	1	21	67	10	33	6	33	91	3	9	67	54	80	13	20			
	11/ 6/36	44	15	35	29	65	117	26	22	91	78	161	41	23	120	75			
18 A M	9/ 2/36	19	42	30	107	70	31	7	18	32	82	178	49	22	129	78			
	10/ 2/36	98	37	37	61	63	37	8	21	29	79	135	45	34	90	66			
	11/ 3/36	150	14	9	136	91	60	15	25	45	75	210	29	14	181	86			
19 S C.	9/15/36	24	16	66	8	34	29	16	55	13	45	53	32	60	21	40			
	9/29/36	30	23	76	7	24	25	21	84	4	16	55	44	80	11	20			
	10/29/36	107	19	18	88	82	47	32	66	15	34	154	51	33	103	67			
20 M R	9/18/36	34	29	85	5	15	76	59	79	17	22	110	88	80	22	20			
	9/30/36	15	12	80	3	20	29	18	62	11	35	44	30	70	14	30			
	11/ 5/36	48	29	60	19	40	22	15	08	7	32	70	44	63	26	37			
21 F L	9/17/36	102	42	41	60	59	54	41	75	13	23	156	83	53	73	47			
	10/ 1/36	32	23	71	9	29	19	10	52	0	18	51	37	65	18	35			
	11/ 2/36	31	18	58	13	42	19	11	58	8	12	50	29	58	21	42			
22 B B	9/10/36	22	14	63	8	37	26	6	23	20	77	48	20	41	28	59			
	10/ 1/36	55	46	83	9	17	26	21	80	5	20	81	67	82	14	18			
	10/31/36	68	55	80	13	20	37	28	75	9	25	105	83	79	22	21			
23 T. R. †	9/15/36	8	2	25	6	75	15	7	46	8	34	23	9	39	14	61			
	10/ 5/36	59	42	71	17	29	31	28	90	1	10	90	70	78	20	22			
	11/ 9/36	68	36	53	32	47	167	26	15	141	85	235	62	26	173	74			
24 H E	9/31/36	127	9	7	118	93	377	12		15	97	504	21	4	483	96			
	10/ 5/36	23	12	52	11	48	14	12	85	2	13	37	34	64	17	36			
	11/ 5/36	94	76	80	18	20	90	36	40	54	60	184	112	60	72	40			
25 E D	9/21/36	10	14	87	2	13	18	15	83	3	17	34	29	85	5	15			
	10/ 1/36	21	20	95	1	5	11	11	100	0	0	32	31	97	1	3			
	10/31/36	80	27	46	43	54	36	17	47	19	53	116	34	46	62	54			

ject R W, who had 94 per cent of cornified cells in the right eye and 16 per cent in the left, a difference of 78 per cent. Sixteen, or about two thirds, of the subjects showed a difference between the right and left eyes of ten or more, on one or both smears made before administration of supplementary vitamin A.

A similar difference was found in smears taken at different times in the same individual either from a single eye or in the combined smears from the two eyes. In smears from either eye singly differences as great as 82 per cent (H E) were found, and the combined smears showed a maximum difference of 60 per cent (H E). Eleven of the subjects showed a difference of 20 or more per cent in one eye or the other, and 12, or about half, in the combined smears in successive examinations showed as great a difference.

When the results of the examination of smears before and after the administration of vitamin A are compared, it is found that in general there appears to be a decrease in the number of cornified (nonnucleated) cells. The average number of cornified cells in the combined smears after vitamin A was 39.2 per cent, and the median 36 per cent. Also, twenty of the twenty-five subjects had fewer cornified cells in the combined smears after taking the added vitamin A than before. However, when the eyes are considered singly, it was found that only eight of the twenty-five had fewer cornified cells in both eyes considered

separately, while seventeen actually had as many or more. Furthermore, the differences in the per cent of cornified cells before and after added vitamin A were no greater than the differences in successive smears or between the two eyes before the vitamin was given. Finally, seven of the supposedly normal subjects were later found to have evidence of vitamin A deficiency as shown by the photometer. Yet they showed no more significant differences in eye smears before and after A than those who had normal photometer readings.

The results in nine patients, three of them children, in whom smears were obtained before and after the administration of vitamin A were entirely similar to those obtained in the normal subjects (Table II). The average per cent of cornified cells in combined smears from both eyes before vitamin A was given

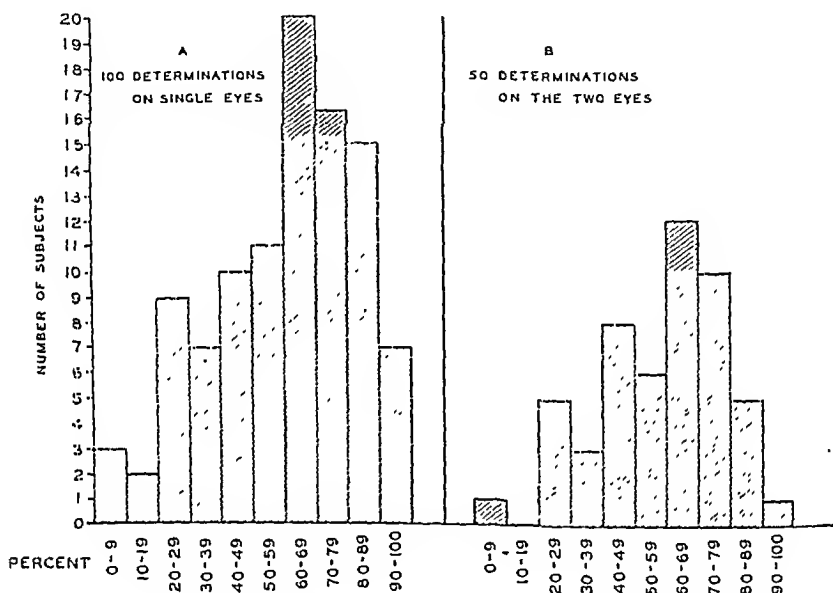


CHART I DISTRIBUTION OF PERCENTAGE OF CORNIFIED CELLS IN "NORMAL" INDIVIDUALS. (BEFORE ADMINISTRATION OF VITAMIN A SUPPLEMENT.)

was 58.8 per cent, with a range of 18 to 90 per cent. The same variations between the two eyes and between successive smears were noted. Of the nine subjects four showed as many or more cornified cells in the combined smears after vitamin A as before, and only two of these showed other evidence of vitamin A lack. The four patients with other evidence of vitamin A deficiency did not show as great a difference, on the whole, as the remaining five. Single smears from 36 patients who received no added vitamin A exhibited no significant difference from the smears from normal persons.

DISCUSSION

The fact that large numbers of cornified epithelial cells may be found in smears from the bulbar conjunctivae of normal subjects and that variations in successive smears or between smears from each eye are as great as the change following the administration of vitamin A in adequate dosage leads to the con-

TABLE 11

EPITHELIAL CELLS IN SMears FROM THE CONJUNCTIVAE OF NINE PATIENTS BEFORE AND AFTER RECEIVING SUPPLEMENTAL VITAMIN A

SUBJECT	DATE	RIGHT EYE						LEFT EYE						COMBINED EYES					
		TOTAL CELLS	CORNI FIED	NORMAL	TOTAL CELLS	CORNI FIED	NORMAL	TOTAL CELLS	CORNI FIED	NORMAL	TOTAL CELLS	CORNI FIED	NORMAL	TOTAL CELLS	CORNI FIED	NORMAL	TOTAL CELLS	CORNI FIED	NORMAL
1. A. W.	7/20/36	50	NO	0%	NO	0%	NO	50	NO	0%	NO	0%	NO	50	NO	0%	NO	0%	NO
	7/27/36	26	8	31	18	69	51	20	39	31	61	77	28	36	49	64	28	36	49
	8/17/36*	20	11	55	9	45	6	4	66	2	34	26	15	60	11	40	15	60	11
2. P. C.	9/26/36	30	21	70	9	30	26	13	50	12	50	56	34	60	22	40	56	34	60
	10/ 3/36	116	1	26	85	74	56	14	60	22	40	172	65	38	107	62	172	65	38
	10/30/36	17	13	76	4	24	44	28	63	16	37	61	41	67	20	33	61	41	67
3. A. P.	9/26/36	70	34	48	36	52	28	21	75	7	25	98	55	50	43	44	98	55	50
	10/10/36	78	24	63	14	37	31	26	83	5	17	69	50	71	19	29	69	50	71
	11/14/36	8	4	50	4	50	2	2	100	0	0	10	6	60	4	40	10	6	60
4. N. E.	10/ 3/36	50	31	62	17	34	50	27	54	23	46	100	60	60	40	40	100	60	60
	10/10/36	50	31	62	19	38	26	1	50	13	50	76	44	58	32	42	76	44	58
	11/21/36	21	12	57	9	43	23	2	8	21	92	44	14	32	30	68	44	14	32
5. W. T.	10/ 3/36	30	6	20	24	80	63	11	17	52	83	91	17	18	76	32	91	17	18
	10/10/36	170	343	92	20	8	142	66	89	56	11	712	647	90	65	10	712	647	90
	11/28/36	56	27	41	29	56	114	21	18	9	72	170	48	25	122	75	170	48	25
6. B. H.†	10/14/36	29	19	65	10	35	25	24	96	1	4	54	43	80	11	20	54	43	80
	11/13/36	31	23	74	8	26	3	5	24	27	76	64	31	48	53	52	64	31	48
7. W. W.	10/17/36	38	35	92	3	8	140	75	5	6	47	178	110	62	68	38	178	110	62
	10/21/36	193	66	34	127	66	111	99	87	14	13	306	165	53	141	47	306	165	53
	11/21/36	18	10	56	8	44	19	9	47	10	53	17	19	51	18	40	17	19	51
8. V. T.	12/ 1/36	1	27	87	4	13	40	22	5	18	45	71	40	69	22	31	71	40	69
	1/ 6/37	85	40	47	45	53	40	1	85	6	17	123	74	59	51	41	123	74	59
	2/ 2/37	19	1	68	6	32	16	6	78	10	62	15	19	74	16	40	15	19	74
9. E. W.†	12/ 1/36	119	82	60	37	40	100	95	95	5	5	219	177	80	42	26	219	177	80
	1/ 8/37	20	18	90	2	10	45	29	64	16	36	65	47	72	18	28	65	47	72

*In each case, the first two readings are before and the third after

†Only one preliminary smear was obtained

clusion that the presence of such cells, even in considerable numbers, cannot be taken as satisfactory evidence of a mild vitamin A deficiency in the adult. The results of an examination of patients, some of whom presented other evidence of mild A deficiency, support this conclusion, as does the fact that seven supposedly normal subjects, who by other tests (photometer) gave evidence of mild deficiency and improved under treatment, had no more cornified cells than others who failed to show this evidence of deficiency. The only factor likely to weaken this conclusion is that of time. Treatment was continued for only one month. It is known that changes resulting from vitamin A deficiency in at least one other epithelial surface, the skin, may require a much longer time than this to disappear (thus differing from hemeralopia or night blindness which may be relieved very quickly). Therefore, it is possible that a longer period of treatment would have caused a greater and more consistent change in the character of the cells in the smears. This appears unlikely.

It is undoubtedly true that cornification and other changes in the cells of the conjunctiva occur in vitamin A deficiency. Pillat,* in particular, has made an exhaustive study of material obtained from the cornea and conjunctivae in

cases of vitamin A deficiency and has demonstrated characteristic changes in the epithelium, including the presence of various bacteria within the cells. Furthermore, the changes have been observed long before the development of keratomalacia or even of xerophthalmia. They are present in the stage which he and others have termed prexerosis. However, changes of the grade of even prexerosis are indicative of rather severe deficiency according to present standards which, based on such a method as the visual photometer, or in some cases the presence of a specific dermatosis, may diagnose vitamin A deficiency before prexerosis appears. Hence changes in the epithelium occurring at the stage of prexerosis are unsuited for our purpose. Therefore, it is probable, that while the presence of cornification in large numbers of cells (nearly all) in smears from the eye on successive examinations may be taken as suggestive of vitamin A deficiency, particularly if the condition is relieved by treatment with vitamin A, it is also probable that other and more obvious evidence of the disease would be present and that the deficiency might have been detected much earlier by other means.

SUMMARY AND CONCLUSIONS

Examination of smears from the bulbar conjunctivae of normal subjects and 9 patients, before and after the administration of vitamin A in large doses, revealed large numbers of cornified epithelial cells. Great variations were noted in successive smears and in the smears from each eye. No consistent difference was noted after administration of vitamin A. The presence of even large numbers of cornified cells in smears from the conjunctivae cannot alone be taken as evidence of early vitamin A deficiency in adults.

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TRICHINOSIS DISTRIBUTION OF TRICHINELLA SPIRALIS IN PORK PRODUCTS SOLD IN PHILADELPHIA*

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SPORADIC outbreaks of trichinosis involving numerous individuals in scattered communities together with improved methods of examination of material for encysted parasites and newer diagnostic tests have stimulated investigators to give considerable attention to this disease in recent years. Many have emphasized its importance as a significant health problem in the United States, a disease often undiagnosed because of its frequent atypical manifestations.

The causative organism is a small roundworm considered for many years as primarily a parasite of rats. It was believed in the past, and there are still many supporters of the view today that horses from which the parasites are generally obtained by man became infested as a result of eating trichinous rats.

Most human outbreaks which have been thoroughly investigated have been traced to infested pork although in one particular case at least, the outbreak was traced to infested bear meat.¹ Birds, herbivorous animals, and cold blooded animals do not normally harbor encysted parasites.

The parasites enter man and other hosts as larvae encysted in the striped muscle of meat foods. They are usually most abundant in the pillars of the diaphragm, the tongue, the abdominal and intercostal muscles, the psoas muscles and the muscles of the back. Ingestion of unskilled larvae in poorly cooked meat results in their liberation by the digestive action of the gastric enzymes. The larvae mature in the intestine in two to five days, and during a period of five to eight weeks a female which is viviparous may deposit 1,500 to 10,000 embryos.² The adult male worms and finally the females, are passed off from the body. The embryos penetrate the wall of the intestine and pass primarily to the striped muscle in different parts of the body, although they have been found in other tissues including the brain.^{3,4} They pass from the intestine by way of the lymphatics and are then transported by the blood. On rare occasions they have been isolated from fresh samples of blood after taking with 3 per cent acetic acid.^{3,4}

The embryos become encysted in the muscles and complete their larval development by the end of the third month.² After some time the cyst undergoes calcification and degeneration, and if the host survives up to this point the symptoms generally disappear. In answer to a query the editor of the Journal of the American Medical Association⁵ states that it is unlikely that symptoms recur four years after a typical infestation because of the rapid

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calcification of the cysts. Underhill,² on the contrary, refers to a case in which, after eleven years, the trichinella capsules were not completely calcified and contained larvae still capable of producing experimental trichinosis.

Clinically, the symptoms follow closely the progress of the parasites in the body. Gastrointestinal symptoms appear in twenty-four to forty-eight hours after ingestion of the infested meat and persist from one to ten days. Coinciding with invasion of the voluntary muscles, the patient generally experiences on the seventh to tenth day either extreme muscle pain, edema of the face and eyes, or both, and at times chills and a temperature ranging from normal to 104° F.⁶

Absolute proof of the presence of the infestation depends upon the demonstration of parasites by muscle biopsy, usually from the deltoid or gastrocnemius. There is often a leucocytosis of 15,000 to 30,000, and in typical cases a relative eosinophilia as high as 80 per cent, and rarely less than 15 per cent.⁷ Spink and Augustine¹³ reported an eosinophilia in one case as high as 89 per cent. Eosinophiles have been demonstrated in the stools.²⁵ Diagnosis may often be hampered because of a delayed appearance of eosinophiles in the stained smear. Merritt and Rosenbaum,⁸ reporting on cases of trichinosis involving the nervous system, state that in a serious case under their observation eosinophiles were absent in a stained smear examined more than two weeks after the onset of symptoms, but appeared in the next daily examination and every day while the patient remained in the hospital. Ten weeks after appearance of the eosinophilia (two months after discharge from the hospital) the blood of this patient still showed an eosinophilia of 14 per cent.

Cases of severe trichinosis have at times been reported where there has been an absence of eosinophilia, possibly due to an incomplete follow-up with a sufficient number of blood studies. Not uncommonly patients critically ill are admitted to hospitals showing at the onset no eosinophilia, or at most 1 or 2 per cent. Although other laboratory tests have been devised as aids in the diagnosis of trichinosis, a relative eosinophilia, with a history of eating pork, is reasonable evidence to support a suspicion of trichinosis.

Complement fixation, precipitin, and skin tests have been devised and used in the diagnosis of trichinosis. Bachman¹¹ described an intradermal reaction in infested laboratory animals and a precipitin test somewhat earlier. The antigen used is prepared from dried trichinella larvae, and it has been employed by a number of workers with variable success. Some are very enthusiastic about its value and consider it within the range of reliability of most laboratory determinations. McCoy, Miller, and Friedlander¹² found the intradermal reaction positive in 90 per cent of eighty-eight known cases of trichinosis. Spink and Augustine¹³ found 33 positive skin tests out of 34 tested. Drake, Hawkes, and Warren¹⁰ got 24 positive reactions in 26 individuals ill with probable trichinosis, and conclude that the reaction is positive in individuals who are or who have recently been ill with trichinosis, and that negative results may be obtained in latent cases. Heathman¹⁵ found the precipitin and skin tests of little value as compared with the older established laboratory determinations.

Spink points out in a more recent publication¹⁴ that discrepancy in results obtained by different workers in the use of the intradermal test and precipitin reaction has been due to a lack of familiarity with the different types of skin responses produced and to the error in comparison of human skin responses with those of laboratory animals. With 25 additional cases, bringing the total under his observation to 60, 59 gave positive skin reactions. The negative reaction was obtained in a patient in a moribund condition definitely due to trichinosis. He emphasizes that there are two distinct skin reactions obtained with the trichinella antigen. There is an immediate reaction, usually not obtained until the second week of the infestation, which is characterized by the appearance within five minutes of a blanched wheal, at times with radiating pseudopodia. There is a pronounced area of erythema surrounding the wheal. This reaction reaches its maximum in one hour, then gradually subsides. It is this reaction that may be found persisting for months and years after subsidence of the acute attack.

The second reaction is delayed and is observed only early in the disease. It is characterized by an immediate erythematous flare which subsides quickly. Then in twelve to twenty-four hours and reaching a maximum in eighteen to twenty-four hours, there appears at the site of the injection a reddened, slightly edematous and tender area from 1 to 3 cm in diameter. The reaction gradually subsides over a period of days, and is replaced later in the course of the disease, during the second week of the patient's illness, by a reaction of the immediate type.

Spink found the precipitin test positive in all cases performed with serums of 35 patients ill with trichinosis. The precipitins usually did not appear until the fourth week of the disease but were demonstrated as early as the second week. On the basis of four years' experience he recommends that these tests be used routinely in the diagnosis of doubtful cases of trichinosis.

It is interesting that in cases of suspected trichinosis with a history of eating pork, detailed investigation of other individuals present and eating the same meal has frequently disclosed positive laboratory evidence of trichinella invasion in many or most of them even though they may not have developed typical clinical symptoms. Merritt and Rosenbaum,⁸ investigating one of their hospital patients, learned that she together with three friends, had eaten pork patties at a local restaurant. One of the three friends was ill for two days while the other two experienced no ill effects whatsoever. The one who was ill showed an eosinophilia of 40 per cent and the other two, 8 and 9 per cent, respectively. Drake, Hawkes, and Warren¹⁰ investigating an epidemic in Maine, found that 25 of 71 persons who had eaten infested pork, showed eosinophilia without other signs or history of trichinosis. Otto and Janney⁹ have recently published a very interesting study of an outbreak of trichinosis in a Maryland family. Twelve persons were involved in this particular outbreak, and all gave the history of eating poorly cooked pork shown by subsequent examination to be infested with living trichinella larvae. Three of the individuals were not even mildly ill. The conclusion is drawn that although differences in the degree of illness might be due to differences in natural resistance, it is more probably due to

ingestion of varying numbers of living larvae. This is readily understandable, since some portions of a large piece of pork often are cooked less thoroughly than the bulk of the meat, and the person eating the rarer portion is likely to get more of the living parasites.

Other factors undoubtedly enter. The portion of the meat eaten unquestionably influences the number of worms ingested, since the parasites display a predilection for certain muscles of the body, and particularly those parts near the tendinous insertions. In ground pork and in the so-called "country" or fresh sausage, as will be shown later, the muscle tissue may be so diluted with fat that it is readily conceivable that a number of individuals might eat from the same sample of food with only very few ingesting muscle fibers containing larvae. It must also be remembered that in a sample of meat there might be larvae in varying degrees of degeneration and calcification. Some of the parasites are undoubtedly less resistant to the gastric juices while others in all probability are expelled from the intestine before sexual development. The number of parasites in the muscles of the human host does not depend directly upon the number ingested with the food. Those taken in with the food do not themselves leave the digestive tract to penetrate the tissues of the body. Only the larvae produced sexually in the human host by mature females, may enter the circulation and pass to various parts of the body.

Trichinosis has until the last few years been considered a relatively unusual disease in the United States, and for that reason alone has rarely been considered in differential diagnosis. Outbreaks have been recorded in the literature from time to time, and the United States Public Health Reports list a number of cases yearly. Infestation with trichinellae, with or without clinical symptoms of trichinosis, must, however, be considered very prevalent in the United States, as evidenced by the recent postmortem examinations of individuals who displayed during life no symptoms of trichinosis and whose conditions were never diagnosed as trichinosis.

McNaught and Anderson¹⁶ in San Francisco, examining 200 human diaphragms obtained at autopsy, found by the digestion method with artificial gastric juice, 24 per cent infested with *trichinella spiralis*. All of the positives disclosed living larvae. An additional 25 diaphragms from newborn infants were negative. For none of these cases was there a clinical record of trichinosis. Differential leucocyte studies on stained blood smears were available in 58 per cent of the positive cases, with 4 per cent as the highest eosinophile count. In a case where 3,800 larvae were found in 50 grams of diaphragm, no eosinophiles were found in the differential count, and the authors conclude that the normal eosinophile counts probably indicate long-standing or light infestations. Although a large incidence is indicated by this work in San Francisco, there is no correlation with statistics of the Department of Health of San Francisco, which leads to the conclusion that most of the infestations result in no severe manifestations or go unrecognized.

A number of other workers, by various modifications of similar techniques, have recorded incidences ranging from 3.5 to over 27 per cent. Hall and Collins¹⁷ studied material obtained from 300 cases in hospitals in and around

Washington, D. C. Their examinations were made by digestion and by the microscopic examination of pressed specimens. From their observations they conclude that findings of previous workers are undoubtedly too low. They found 41 or 13.67 per cent of their 300 examinations to be positive. In their series of positives none had a history of any previous diagnosis of trichinosis, although in one case there were between 900 and 1,000 larvae per gram of diaphragm muscle. According to their calculations there are probably several million people in the United States infested with trichinellae, with several hundred thousand who have had clinical trichinosis never diagnosed as such, and possibly several thousand deaths annually from this cause. Although they found living trichinellae predominating in light infestations, and dead trichinellae predominating in heavy infestations, and suggest that the rapidity of calcification may be proportional to the intensity of infestation, it would seem necessary to bear in mind that an individual over a long period of time might be subjected to a number of different infestations, and that the older parasites from the older infestations are continually degenerating. What appears then to be a heavy infestation, with the majority of larvae calcified and dead, might be in reality a cumulative result of a number of infestations. From the prevalence of living parasites in pork products as will be shown later, a number of different exposures by the same person seems very probable.

It appears to some that an infestation might result in some sort of immunity to subsequent infestation of the tissues by the parasites.¹³ The establishment of some type of immunity in infested animals is indicated by investigations by the United States Bureau of Animal Industry. An extensive series of tests showed that filtered artificial gastric juice in which trichinous meat had been digested contained a principle that serves as a partial immunizing agent when swallowed by rabbits and other small mammals. A filtrate obtained from digested noninfested meat did not display these substances which hinder the development and survival of the parasites.¹⁴

Believing that a knowledge of the degree to which pork products are infested with living trichinellae would be an interesting and valuable preliminary to the study of the prevalence of the parasite in human hosts in Philadelphia, a study of meat offered for sale over the counter in representative local stores was undertaken. Up to the present 150 samples of pork have been examined and the study is to be continued. An attempt is being made to determine whether meat from certain particular sources of supply is more frequently infested than from others, and which of the commonly sold pork products most often contain parasites. The samples analyzed were of three kinds, pork chops, fresh "country" sausage, and "homemade" Italian sausage, the latter being included because it is frequently eaten raw.

Sixty-four samples of pork chops, 63 samples of fresh country sausage, and 23 samples of fresh Italian sausage, of all price grades, were analyzed. The samples were obtained during four months of 1936-37 from stores in different parts of Philadelphia. The meat, with the exception of the Italian sausage, represented the products of 20 producers, both small local firms and large national meat packers. Fifty samples were made by two packers supplying

a great bulk of this meat product in Philadelphia. Twenty-three samples were of one local packer supplying a chain of grocery stores in the city.

METHOD OF ANALYSIS

The samples were analyzed by a modification of the digestion method employed by recent workers in the examination of human diaphragms. The digestion mixture consisted of 1 per cent pepsin (1 to 3,000 grade) solution in 0.7 per cent hydrochloric acid. Fifty grams of a sample were digested in 400 c.c. of digestion mixture.

The digestion apparatus consisted essentially of a constant temperature water-bath provided with mechanical stirrers. Our apparatus was similar to the malt mashing machines on the market and accommodated five pyrex beakers stirred by five glass stirrers at 90 revolutions per minute. The bath was maintained at 37° C.

The digestion mixture was made up as used. All apparatus was thoroughly cleaned to prevent contamination of subsequent samples.

Pork sausage, which is already thoroughly ground, was merely removed from the casing, and 50 grams were placed in the digestion mixture. Although some workers have found digestion in an incubator satisfactory for diaphragm samples, stirring for considerable periods of time was necessary here, due to the great amount of fat present in the fresh sausage samples. As digestion progressed, the fat separated as a supernatant layer, and the amount present in some samples was really astounding. Four or five hours of digestion seemed adequate for most of the samples, although much longer periods seemed to have little effect on the living parasites present. When digestion was complete, the stirrers were removed and the beakers were allowed to stand in the water-bath for an additional fifteen to thirty minutes. They were then removed and the layer of fat was carefully skimmed off. Incidentally, the beakers used were of the tall variety without lips, and the sediment was not agitated by this procedure. The contents was then thoroughly agitated with a spoon and poured through an ordinary tea strainer of about 18 mesh to the inch, into a six-inch funnel provided with a pinchcock. The material in the funnel was slowly stirred from time to time, permitting the particles adhering to the side of the funnel to settle. After an hour's standing, small quantities of sediment were taken at a time in a Petri dish for examination under the low power of the microscope. If the sample proved negative, about 10 c.c. of material were examined; if positive, successive portions were examined until two portions were negative. Generally, all of the parasites were found in the first few portions of sediment. In all cases of positives, parasites were found in the first portion.

Pork chops were used which were large enough to provide 50 grams of lean meat. The fat was cut away from the meat, and the meat was then ground coarsely in a meat grinder. By this procedure, practically complete digestion was made possible, and there was hardly any fat to be skimmed after digestion. Examination was as above.

Fresh Italian sausage was also ground, since it contained large masses of cartilage and other material. Apparently in the manufacture of much of this

latter food, the ingredients are not ground but cut into cubes. Much of the material consisted of indigestible animal tissue and plant seasoning.

FINDINGS

Of the 63 samples of sausage, living trichinellae larvae were found in 6, or in 9.52 per cent. Of the 64 samples of pork chops, 1, or 1.56 per cent, were positive. No living parasites were found in any of the samples of Italian sausage. For the present, the findings in the analyses of the Italian sausage may be considered of no great significance, due to the small number analyzed, and due to the fact that it is not a commodity purchased by many of the residents of this city. Of the 7 positives, 2 were found in 17 samples of sausage from one large packer who, as we have been informed by a representative of the United States Department of Agriculture, handled Western hogs. This is the equivalent of 11.8 per cent positive. Twenty-two pork chops from this packer were negative. Three samples of sausage and three of chops produced by a small local concern gave 1, or 33 per cent, positive in each group. Of 17 sausage and 6 pork chop samples produced by a packer in the environs of Philadelphia, 2 of the sausage samples, or 11.8 per cent were positive. Of 7 sausage samples of another small local concern, 1, or 14.3 per cent, were positive. Thus all positives were found in meat from 4 out of 20 packers. The findings are tabulated below.

DISTRIBUTION OF POSITIVE SAMPLES

PACKER	SAUSAGE SAMPLES EXAMINED	POSITIVES	CHOP SAMPLES EXAMINED	POSITIVES
1	17	2 (11.8 per cent)	22	0
2	3	1 (33.3 per cent)	3	1 (33.3 per cent)
3	17	2 (11.8 per cent)	6	0
4	7	1 (14.3 per cent)	0	0

A very noteworthy finding was the small number of parasites in most of the positive sausage samples. Five parasites were found in one sample, two were found in each of three samples, and only one was found in each of two samples. Analysis of the positive pork chop disclosed 15 living larvae. This may be readily explained by the fact that sausage besides being diluted with considerable fat and seasoning, is the product usually of meat from a large number of individual hogs. Whereas a sample of lean meat from a badly infested hog might show a large number of parasites, sausage made in one of the packing establishments from a portion of this meat would undoubtedly show a considerably smaller number. Not only does this practice explain the small number of parasites in individual samples of sausage, but it also indicates the reason for the high incidence in sausage samples as compared to chop samples.

In all cases the parasites were typical in morphology, slightly active, and recognizable without difficulty. The figures given above do not indicate the number of meat samples obtained from infested hogs, but only the number of ~~and~~ tanning living larvae capable of producing trichinosis in man. Degenerated and dead encapsulated larvae were destroyed during the digestion process. Examination of the same samples of meat by other methods might show in addition to

parasites capable of enduring the digestion process, others in a dead or less resistant condition. The calculated number of living larvae per pound of sample was 135 in the case of the positive pork chop, and between 9 and 45 in the sausage samples.

DISCUSSION OF FINDINGS

The finding of living trichinella larvae in fresh samples of pork is not astonishing to those familiar with the life history of the parasite. Government Departments have on numerous occasions emphasized in publications the necessity of thoroughly cooking pork in order to insure the death of the parasites. Placards have been offered to packers and dealers for display in stores cautioning the public, but it can be safely stated that no reader of this paper has ever seen one displayed. The public, to the contrary, has been led to believe that the Government is always on the lookout for harmful meat, and that Government inspection implies approval of the product in all respects. An average layman is astonished and skeptical when informed of the presence of parasites in pork, and almost always expresses the belief that all meat is inspected by the United States Government, and that inspection includes examination for the trichinella parasite. There is at present a large amount of meat sold in this country which undergoes no inspection, and none at the present time is examined for trichinellae. Microscopic examination of about 8,000,000 hogs in the United States between the years 1898 and 1906, when inspection for the parasite was compulsory in pork intended for export, showed between 1 and 2 per cent were positive.¹⁸

Examination of 4,740 samples of pork from grain-fed hogs was made by the Bureau of Animal Industry, by the digestion procedure, with about 1 per cent showing infestation with trichinellae. Two thousand three hundred and forty-one samples of pork from garbage-fed hogs, on the other hand, showed a 5 per cent infestation.¹⁹ These figures coincide with those obtained in a similar investigation and reported in 1935. Although there is no regular inspection or control of all pork products in establishments operating under federal supervision, pork products customarily eaten raw undergo special processing. Three thousand two hundred and fifty-four official samples of pork products so processed were also examined in the above investigation, and the processing was found adequate to destroy the vitality of the parasites.²⁰

Trichinosis is a disease for which there is at present no cure, and all measures undertaken must of necessity be preventive. Since there is also at present no routine examination in this country of pork products for trichinellae, education of the public on the dangers of raw or inadequately cooked pork seems imperative. In New York City, over a period of five years, 166 cases of trichinosis were reported as such. Of these about 65 per cent occurred among Italians and Germans, peoples accustomed to eat pork products raw. Of this group of 166, 14 of 52 individuals who had eaten pork sausage had eaten it raw, and 15 of 86 cases with a history of eating fresh pork admitted eating it raw.²¹

Adequate control of the feeding of hogs would seem from recent observations to be another effective preventive measure. The importance of the rat as a

reservoir of trichinellae parasites is being minimized by the observation of the high incidence of infection in garbage fed hogs. As has been stated above, hogs fed on garbage, which frequently contains raw meat scraps, have been shown to have about five times as high a percentage of infestation as the others. Drake and his associates¹⁰ found an outbreak due to the eating of pork from hogs fattened on garbage. Ott and Janney traced an outbreak in a large family to similar hogs.⁹

The pork in a large city like Philadelphia comes from a great many sources. Much of it apparently is obtained from western corn fed hogs. There are, on the other hand, packing plants which undoubtedly handle local hogs collected from various sources and reared under varying conditions. There are neighboring farmers who handle among other farm products, "home prepared" pork products in their local stalls. Not far from Philadelphia all of the garbage from a large boys' school is collected and fed to hogs in a neighboring piggery. Garbage is regularly collected in Philadelphia and transported to New Jersey for hog food. Ashbrook and Wilson²² state that of 610 cities, with populations of over 10,000, 35.3 per cent disposed of garbage by feeding to hogs during 1916, and calculate that about 40,000,000 pounds of garbage fed pork is sold each year. Although producers are advised in a Government pamphlet that raw garbage is a better hog food than cooked garbage, later publications are suggesting the cooking of garbage before feeding to hogs.

The incidence of trichinellae in hogs must be considered as very significant in view of the severity of the disease in man, and the food habits and ignorance of the disease among so many of our population. The number of cases in man, as has been pointed out, cannot be judged by the number of cases that have been reported. In the past the disease has rarely been considered in diagnosis and almost as rarely identified. The sporadic cases and the milder forms often pass unrecognized. Spink and Augustine¹³ observed 35 cases of sporadic trichinosis during a three year period, and the preliminary diagnoses after a history had been taken and a complete physical examination done, with laboratory data incomplete, were trichinosis in 11 cases, with 'upper respiratory infection,' typhoid, pelvic inflammatory disease, poliomyelitis, gastroenteritis, and tuberculous meningitis predominating in the others. Among the early false diagnoses reported by Otto and Janney were typhoid fever, influenza, and mumps.⁹

Pork as now sold is a definitely dangerous food unless thoroughly cooked. The pork samples analyzed contained few parasites, but undoubtedly sufficient to produce after reproduction in the intestine, the mild or subclinical type trichinosis. The severe textbook type of the disease is undoubtedly due to massive infestation with parasites. Chandler²³ mentions the finding of over 2,000,000 larvae per pound in sausage responsible for an outbreak of trichinosis in Portland, Oregon. No sample in the present study approached this number but there is, however, little doubt that very badly infested meat has been sold in Philadelphia in the past and with the prevailing ignorance of the danger of the disease, will continue to be sold and eaten in a raw or poorly cooked condition.

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THE CORRELATION OF CLINICAL DIAGNOSIS AND POSTMORTEM FINDINGS IN TRICHINOSIS*

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TO THE average physician, trichinosis is a parasitic disease infrequently seen and rarely diagnosed. Since this public enemy attacks man under the "red flag" of fever, its onset and course are frequently mistaken for an attack by bacterial agents. Trichinosis has been recognized with increasing frequency as it has received increasing attention. The method of ascertaining the incidence of trichinosis in man which has given the most precise information to date is the study of necropsy and dissecting room material. Hall and Collins recently summarized the postmortem studies made in the United States¹. Two hundred twenty two in a total of 1,778 cadavers examined, or 12.5 per cent, had trichinae. Hall and Collins further point out that by the use of two techniques in these examinations, the direct microscopic and the Baerman method, the incidence would probably have been close to 17.5 per cent.

The symptoms of trichinosis depend in large part on the number of parasites present and, in the absence of an epidemic, the highly variable symptomatology may suggest some other disease. When few adult worms develop, the small number of embryos which reach the muscle may cause little, if any, reaction on the part of the host. On the other hand, with large numbers of parasites severe symptoms and, in some cases, death may follow.

In the initial stage the parasite invades the digestive tract of the host and its presence is manifested by nausea and vomiting, usually with watery diarrhea, but sometimes with constipation. Periodic abdominal pain may appear at the end of this stage.

In the second stage, the period of invasion of muscles and other tissues, there is an onset of myositis evidenced by muscular tenderness, which is most marked in the muscles of the extremities. Commonly, the muscles of mastication, speech and respiration are involved. Dyspnea is a prominent feature. Involvement of the muscles of the neck leads to a spasticity and rigidity producing a positive Kernig's sign. Periorbital edema is probably the most commonly recognized and most constant of the various signs and symptoms of trichinosis. Its presence with certain associated optic changes, has been considered by ophthalmologists sufficient evidence for a positive diagnosis of trichinosis. Passage of trichinae to the brain stem, with lodgement in the meninges, may simulate a meningitis, pachymeningitis, or epilepsy, with convulsions as a prominent feature². Transient scarlatiniform rashes are frequently observed. There are often symptoms

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suggesting lung involvement. The temperature varies with the severity of the attack, but often reaches 105° F. or higher, and in character resembles that of typhoid fever. As the disease progresses the patient becomes cachectic and anemic. At this time there may be delirium, somnolence or insomnia, suggesting some acute septic condition. Furunculosis, pleurisy, and pneumonia are common accompaniments.

The third stage, or the period of encystment, corresponds to the period of convalescence and is very prolonged, at times extending well over a year. The most rapid calcification observed in experimental animals, which had been given viosterol to hasten the process, required six weeks.

The diagnosis of trichinosis is made in various ways. Clinical symptoms may suggest trichinosis, or an incidentally discovered high eosinophilia may be the first clue to the diagnosis. The clinical picture is highly variable and may be confused with many other conditions. The eosinophilia is not diagnostic since it occurs in many other conditions and, in trichinosis itself, the eosinophilic cells may be absent in a few cases during certain stages of the period of invasion and may be markedly reduced or entirely absent in many cases in which the condition is complicated by bacterial disease.

One method of confirming a diagnosis is examination of a section harpooned from a voluntary muscle, such as the deltoid, pectoralis major, the outer head of the gastrocnemius, or the lower portion of the biceps. This operation, previously done without anesthesia but now done under local anesthesia, is unpleasant and most patients refuse to permit it. The present tendency is to do a biopsy by incision with removal of a bit of muscle. Many times this procedure is not conclusive since the muscle removed may show no trichinae, even though the parasites are present elsewhere. The muscle tissue should be examined as a press preparation between glass slides and should not be sectioned. In the early stages of invasion, before encystment, the larvae are frequently missed by simple microscopic examination unless the technician has been especially trained in their identification.

The precipitin reaction is very useful in detecting active or recent cases, but as the reaction becomes negative in cases of somewhat less than one and one-half years' duration, it is of no value in detecting old infestations. It is not a widely usable method or a bedside procedure as it requires specialized laboratory facilities and technicians with immunologic training.

Of the available diagnostic methods, the intracutaneous skin test of Bachman is the easiest to perform, is fairly accurate when properly carried out, and gives a rapid method of diagnosis. It has the advantage of detecting old cases. In this study this advantage enabled the authors to check their findings against all positive necropsy findings. The test was introduced by Bachman in 1928,⁴ and was further perfected by Augustine and Theiler in 1932.⁵ As an adjunct to the diagnosis of epidemic cases, reports indicate that the test is dependable in 90 per cent of cases.⁶ This skin test becomes positive within one to two weeks of the time of invasion by the trichina larvae. The duration of skin sensitivity has never been determined, but it is supposed to last at least three to

four years.

In the present paper the writers have undertaken what is, as far as could be determined by a review of the literature, the first study of the skin test in which its response has been correlated with necropsy findings. This study was planned to determine

(1) The correlation between the incidence of trichina infestation, as determined by a routine skin test of patients in Gallinger Municipal Hospital, Washington, D. C., and the incidence as determined by post mortem examination of the diaphragms of the patients included in our series. As a corollary, we hoped to determine the efficiency of the Baehman intracutaneous skin test as a routine procedure in sporadic cases of infestations of varying degrees by comparing the skin test reaction and the necropsy finding of trichinae by microscopic and Baehman examination.

(2) The extent of eosinophile reaction in cases other than epidemic in which trichina infestation is unrecognized in patients hospitalized for other causes, and the relationship of any eosinophilia found to the degree of infestation and to the wheal reaction in the skin.

A reaction was considered positive when a blanched, elevated wheal, with a seemingly flat surface appeared within ten to thirty minutes. Such wheals increase in area more or less rapidly for about fifteen minutes after appearance, by which time maximum dimensions are usually reached and may measure from 10 to 20 mm in diameter. Usually the wheal is more or less regular in outline, but in a few instances it produces narrow pseudopodial outrunners. An erythema, surrounding the wheal, usually develops shortly after the formation of the wheal, and is often quite pronounced within five minutes after the injection. In our series of cases, an antigen of 1:10,000 dilution was used throughout. With this dilution, it was found advisable to read the reaction in fifteen minutes and again in thirty minutes. A wheal under 5 mm was regarded as a negative reaction.

Our studies extended over a period of five months and covered a series of 400 patients. This series included both males and females above 13 years of age, of mixed nationalities but of equivalent social status, practically all of whom would be regarded as "Americans." As regards race the proportion was colored, 264, of which 173 were males and 91 females, white, 136, of which 89 were males and 47 were females. In this study our data were collected and interpreted before the postmortem findings at the National Institute of Health were consulted, to rule out any subjective influence on our interpretations.

Table I gives a summary of the results obtained in this series, showing the incidence of trichinosis as indicated by the skin test and the incidence as obtained on necropsy examination.

The mean incidence, as indicated by the routine skin test of these unselected cases, was found to be 18.25 per cent. This value is very close to that determined for Gallinger Municipal Hospital in a study of the incidence of trichina present in diaphragms on necropsy, the incidence in over 200 cases being 19.1 per cent. This variation in indicated infestation is so slight as to be within the limits of expected variation. There is a theoretical possibility that positive cases not detected by the skin test may be offset by positive cases not detected by postmortem

duced into a susceptible individual. To us, it seems far fetched that there should be a common protein fraction in the nematode and in bacterial agents when they are so far apart phylogenetically. As yet the exact cause of false positives, if this be the explanation in these three cases, is not understood and needs further study. However, we do not feel that the incidence in this series is enough to condemn the test.

Another and more logical explanation of these three cases is that in some instances the infestation may be so light as to be missed entirely on microscopic examination. It is to be remembered at this point that the Baerman examination will only detect viable larvae, hence only rarely those which have been long encysted. Therefore, it will be logical to suppose that, in very light cases, the examination of 1 gm. of muscle microscopically may not be sufficient to detect the presence of trichina in such cases and that the Baerman examination may also be negative. Furthermore, encystment may occur in muscles other than the diaphragm and thus escape detection on necropsy.

The two false negatives are not so easily explained. Here one must consider individual peculiarities in the immunologic response to the presence of foreign protein in the body. It is known that the allergic response in very light infestations is much less than in heavy infestations, and since both cases were of the former character, one yielding one calcified cyst and the other yielding one calcified cyst and one active larva, we may assume that the antigen used was too dilute to detect such light sensitivity. On the other hand, we may also assume that with very light, long standing cases the individual tendency may be to ward a complete loss of this sensitivity.

TABLE II

RELATION OF DEGREE OF INFESTATION, EOSINOPHILIA AND WHEAL REACTION IN POSITIVES

SKIN TEST REACTIONS	TRICHINA/GRAM MUSCLE		AVERAGE EOSINOPHILIA
	MICROSCOPIC	BAERMAN	
Strong 100 mm. over	55	0.87	12.1 per cent
Weak 5 mm. 10 mm.	26	0.03	5.1 per cent

In man most diseases which produce hypersensitivity are also found to produce an eosinophilia. As a separate project, we attempted to correlate the degree of eosinophilia with the extent of the wheal reaction in the skin and with the degree of infestation. Our results are given in Table II.

It is readily seen that the mean values obtained in this series are much lower than the value of 17 to 35 per cent usually stated to be found in chronic cases. The explanation for this marked variation is not as difficult as would seem at first glance. Many of the patients studied were individuals hospitalized for some acute infectious disease. Spink, in 1934, showed that animals infested with trichina larvae would react with high eosinophilia, but that when a second infection was superimposed, the eosinophilic cells present in the enen-

lating blood dropped and, in many cases, completely disappeared. Willet and Pfan carefully followed the blood changes in 14 cases of acute trichinosis and observed that in those cases where superinfection occurred, the eosinophiles disappeared from the blood.⁶

The characteristic hemogram in trichinosis has been traced to the toxic reaction occurring at the site of muscular invasion and has been ascribed to protein metabolism.⁷ When the trichina-infested individual acquires a secondary infection, he has more need for another type of leucocyte, but as soon as the acute infection has subsided the eosinophiles again appear in the circulating blood. Unfortunately, facilities did not permit daily differential counts on each patient. Therefore, we may consider that the eosinophilia represented to a certain extent the response of the patient to the superimposed acute infection and not to the trichina per se.

The relationship of the wheal reaction in the skin to the extent of eosinophilia and to the diaphragm findings was quite consistent throughout the entire series. For proper evaluation and for comparison with the extent of eosinophilia and the degree of infestation, it was necessary to classify the skin reactions as either strong or weak. The former constituted those reactions of 10 mm. and above in diameter, the latter those reactions of 5 to 10 mm. in diameter. On this basis (Table II), it was observed that strong skin reactions were presumptive of heavy infestations, but that in a few cases of high infestation there was poor skin sensitivity so that the wheal reaction was slight. Also, certain cases of light infestation had high skin sensitivity and gave marked wheal reactions.

SUMMARY

In a study, apparently the first of its kind, on the diagnostic value of the Bachman intracutaneous skin test for trichinosis as checked against the post-mortem examination of 30 per cent of the patients skin tested, the results show a close correlation between skin test findings and postmortem findings. The skin test may be somewhat more valuable as a negative diagnostic measure than as a purely positive measure.

A study of the correlation between the extent of the skin test reaction and the degree of eosinophilia in the patient indicated a higher degree of eosinophilia in those cases in which strong skin reaction (over 10 mm. in diameter) was produced.

The evidence indicates that the skin test has a definite value in the diagnosis of trichinosis and that it is to be recommended in cases in which the possibility of trichinosis is considered.

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THE IMPORTANCE OF CONCENTRATION PREPARATIONS OF LEUCOCYTES IN THE STUDY OF THE LEUCOPENIAS*

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THE leucocytes have no important function in the circulating blood. They are present in the blood stream only en route from point of origin to point of localization and activity in the tissues. The cells are counted and studied in a stained film to detect abnormal cell formation or to reveal the need of the tissues for leucocytes and the capacity of the blood forming organs to respond. If the granulocytes overflow normally from the bone marrow the blood study reveals the condition of the marrow. If on the other hand the cells do not freely reach the blood stream a granulopenia develops and it becomes most difficult to visualize the state of the marrow from the usual blood examination. A granulopenia may be due to a toxic myelosis in which the normal maturation and delivery of granulocytes is interfered with a hyperplasia of the leucopoietic tissues characteristic of leucemia, or a simple depression of marrow activity.

A granulopenia is especially apt to occur in acute leucemia when the hyperplasia of marrow is very marked and may be a mechanical barrier to normal cell delivery into the blood stream. In conditions such as pernicious anemia the granulocytes are enmeshed in the hyperplastic red cell forming tissue and a granulopenia results. A granulopenia may also be due to a depression of marrow activity, as in Banti's disease without any qualitative changes in the granulocytes.

A biopsy of the bone marrow may be employed to show the condition of the marrow and aid in making a differential diagnosis. The diagnosis of a leucopenic leucemia or a toxic myelosis and the differentiation of these from the benign leucopenic states depends on having a sufficient number of cells for study.

*From the Cleveland Clinic
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Even a large film does not furnish enough cells to reflect the state of the marrow. A sufficient number can be obtained very simply, however, in every instance.

In 1931 Reich¹ suggested the use of concentrated specimens of leucocytes in studying the leucopenias. Since this procedure was brought to my attention, we have used it routinely in all leucopenias and found it of the greatest assistance in making a correct diagnosis. We have seldom found it necessary to do a bone marrow biopsy to make a diagnosis in such cases. Instead of the few cells available for study in the usual blood film, thousands can be observed in the concentrated specimens.

The preparations are simply made, especially if hematocrit determinations are routinely employed, as is done in our hematology laboratory. Five cubic centimeters of blood are mixed with 1 c.c. of an isotonic anticoagulant (1.4 per cent sodium oxalate) in a Sanford-Magath hematocrit tube. The tube is set aside for ten to fifteen minutes until the red cells settle out by gravity. The sedimentation rate in cases in which this procedure is indicated is usually accelerated so the settling of red cells is rapid. The tube is then spun in a centrifuge at high speed for a few minutes to sediment the white cells and platelets remaining in suspension in the plasma.

With a capillary pipette, specimens from the layer of white cells ("buffy coat") are removed, film preparations are made in the usual way on slides or cover-slips, stained, and mounted. During the short period required for the preparation of the films, no significant change takes place in the leucocytes, so they reflect accurately the state of the marrow.

In a granulopenia due to the effect of amidopyrine on the marrow, striking qualitative changes are detected in the granulocytes, which explain why they are of little value in combatting infection. In the leucopenic leucemias, sufficient cells are always found to reveal the leucemic process in the marrow. In other types of leucopenia, the absence of leucemia or toxic myelosis can be proved. Preparations such as these are also excellent for study of the platelets. A sufficient number of platelets are present in the films to reveal any variation in size or significant qualitative changes.

CONCLUSIONS

Concentration preparations of the white cells should be made in all cases of leucopenia. Such films usually reveal the changes in the marrow responsible for the leucopenia and obviate the necessity for a bone marrow biopsy.

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THE RELATIONSHIP BETWEEN THE CHLORIDES AND THE NITROGENOUS WASTE PRODUCTS IN THE BLOOD*

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THERE has recently appeared a reiteration of the thesis that there is a reciprocal relationship between the blood urea and the blood plasma chlorides¹ Hartmann and Darrow stated their belief in 1928 that loss of plasma electrolyte by faulty urinary secretion is osmotically compensated for by retention of urea² To determine whether there is such a reciprocal relationship, the studies described below were carried out

METHOD

A normal subject, a patient with chronic hemorrhagic nephritis, and a patient with Addison's disease were studied under varying conditions in an effort to determine what relationship exists between the plasma chlorides and the nitrogenous waste products in the blood

The normal subject, a male, aged twenty six years was fed a weighed diet which was the same each day, and 2,600 cc of tap water were taken daily except as otherwise indicated After a three day preliminary period, to allow adjustment to this regime, observations were made for three days to serve as a control (Period 1) During the next ninety nine hours (Period 2) no liquid was ingested except that inherent in the food (417 gm daily) Following this for five days (Period 3) the subject was allowed to drink water freely Period 4 was the second control period During Period 5 the subject ingested 15 gm of urea dissolved in approximately 100 cc of the allotted water during the first twenty four hours, and 45 gm of urea in a corresponding volume of water during the succeeding twenty four hours After Period 6, the third control period, there ensued two days during which 6,000 cc of fluid were taken daily in addition to the diet (Period 7)

Later the same subject was studied in a similar manner, except that the period of dehydration was omitted and the urea feeding consisted of the ingestion of three 20 gm doses during one day Still later he was again studied under the same conditions, except that the period of dehydration extended for eighty four hours

Blood specimens of this normal subject were obtained under fasting conditions every morning, except as otherwise indicated, and were analyzed for urea

*From the Department of Internal Medicine University of Michigan Medical School, Ann Arbor

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TABLE I
FIRST STUDY OF NORMAL SUBJECT

DATE	REMARKS	BLOOD UREA NITROGEN MG./100 C.C.	BLOOD PLASMA CHLORIDES AS NaCl MG./100 C.C.	URINE UREA GM.	URINE CHLORIDE AS NaCl GM.	24 HOUR URINE VOLUME C.C.	OTHER OBSERVATIONS
2/22/36	Period 1: control	10.6	395	12.5	3.3	1055	
2/23/36		8.6	605	10.8	5.0	1635	
2/24/36				10.6	4.0	1320	
2/25/36	Period 2: dehydration	8.7	574	9.4	3.5	530	
2/26/36		15.3	583	10.1	7.0	543	
2/27/36		11.0	661	11.0	7.1	545	
8:00 A.M. 12:00 noon		13.8	635				
5:30 P.M.		16.7	631	16.7			
2/28/36	Period 3: water ad lib.	23.9	687		6.7	490	Hematocrit 48%
8:00 A.M. 5:30 P.M.		24.2	656				
2/29/36		32.9	664		3.0	590	Hematocrit 45% Hemoglobin 94 (S) R.B.C. 5,610,000 per c.mm.
3/1/36 to 3/4/36 inclusive					3.6 5.4	650 930	

TABLE I—Cont'd

3/5/36	Period 4 control	S 8	596	12.6	5.9	1190
3/6/36						
3/7/36						
3/8/36	Period 5 urea ingestion (1.5 gm urea 9:30 A.M.)	8.4	639	22.0	3.5	1460
3/9/36			655			
3/10/36			639			
3/11/36	Period 6 control	21.9	675	46.9	6.2	1710
3/12/36						
3/13/36						
3/14/36	Period 7 "hydration"	8.0	610	4.3	6.8	5210
3/15/36			597			
3/16/36			606			
3/17/36	Period 8 control	14.7	670	18.4	3.6	1470
3/18/36			618			
3/19/36			615			
3/20/36	Period 9 control	9.7	615	12.9	4.3	1600
3/21/36						
3/22/36						
3/23/36	Period 10 control	8.0	610	4.3	6.8	5210
3/24/36			597			
3/25/36			606			

Diet P 70, F 160, CHO 275, Chloride 2.3 gm
 Calories 2838
 H₂O in diet—417 gm
 added—2600 cc.
 added in Period 7—6000 cc

Hematocrit 46%
 Hematocrit 46%
 Hematocrit 48%
 Hemoglobin 97 (%)
 RBC 4,560,000
 per c mm

nitrogen and chlorides.* Twenty-four hour urine specimens were collected (beginning at 8:00 A.M.) and were analyzed for urea and chlorides.†

In the study of the patient with chronic hemorrhagic nephritis‡ fluids were forced to an average of 3,900 c.c. daily, and he was fed an approximately neutral ash diet, yielding about 50 gm. of protein and about 2 gm. of sodium chloride daily, except at one period, during which, for six days, 7.5 gm. of sodium chloride were added to the diet daily. Determinations of plasma chloride and nonprotein nitrogen values of fasting blood specimens were made at frequent intervals during his hospitalization.

Our study of the patient with Addison's disease consisted of frequent, simultaneous chloride and nonprotein nitrogen determinations of the blood during the administration of varying amounts of an extract of adrenal cortex and of sodium chloride.

TABLE II
SECOND STUDY OF NORMAL SUBJECT

DATE	REMARKS	BLOOD UREA NITROGEN MG./100 C.C.	BLOOD PLASMA CHLORIDES AS NaCl MG./100 C.C.	URINE: UREA GM.	URINE CHLORIDE AS NaCl GM.	24 HOURLY URINE VOLUME C.C.
4/19/36	Period 8: control	8.1	579	15.3	2.5	1180
4/20/36		8.3	594	15.7	3.4	1160
4/21/36		8.0	596	16.7	3.9	1700
4/22/36	8:00 A.M. Period 9: urea ingestion (20 gm. urea, 9:00 A.M.; 11:00 A.M., 1:00 A.M.)	8.1	586	62.1	3.8	2290
	6:30 P.M.	37.0	578			
4/23/36	Period 10: control	15.5	610	24.7	4.4	1620
4/24/36	Period 11: "hydration"	9.3	597	19.7	4.8	5390
4/25/36		7.6	597	17.3	5.2	5000
4/26/36		7.7	597			

Diet: P-80, F-150, CHO-300, Chloride 2.9 gm.
Calories 2861
H₂O in diet—1227 gm.
added—1790 c.c.
added in Period 11—5190 c.c.

RESULTS

Results of these studies appear in Tables I, II, III, IV, and V.

The study of the normal subject demonstrated that dehydration to the extent of approximately 6.4 per cent of the body weight (Table I, Period 2) was

*Urea nitrogen was determined by the Van Slyke and Plazin method, and the chlorides by the open Carius method as applied by Eisenman. (Peters, John P., and Van Slyke, Donald D.: Quantitative Clinical Chemistry, Williams and Wilkins Co., Baltimore, 1932, Vol. II, Methods, pp. 558, 837.)

†Urea was determined by the Van Slyke and Cullen urease with aeration and titration method, and the chlorides by the modified Volhard-Harvey titration method (Idem, pp. 547, 833).

‡A male, aged seventeen years, was seen for the first time at the University Hospital in August, 1930, with hematuria and edema following scarlet fever. He was seen on a number of occasions afterwards, and our study of him began in April, 1936. In July, 1936, he was readmitted in uremia and died during his stay in the hospital.

accompanied by a sharp increase in the concentration of blood urea. The concentration of the plasma chlorides was also increased but to a lesser extent. The subsequent dehydration study (Table III, Period 17) demonstrated that dehydration to the extent of approximately 4 per cent of the body weight was also accompanied by an increase in the concentration of the blood urea but to a lesser degree. The concentration of the plasma chlorides appeared not to be greatly altered. Upon rehydration, these blood values returned to normal. During the 3 periods of urea ingestion (Periods 5, 9, and 13) the blood urea nitrogen concentration rose to 26.9 mg, 37.0 mg, and 34.4 mg per cent, respectively, but the plasma chlorides did not fall.

When huge amounts of fluid were ingested (Periods 7, 11, and 15), there was a slight diminution in concentration of blood urea, but there was no consistent change in the concentration of the blood chlorides.

These observations on a normal subject demonstrate that the rise in concentration of blood urea accompanying dehydration is not accompanied by a fall in concentration of the blood chlorides. It might appear that at the peak of dehydration (Table I) the increase in concentration of the plasma chlorides (from 57.4 mg to 66.4 mg per cent) is only an apparent one and is not absolute, and that in actuality, because of the diminution in the volume of extracellular fluid, this value is representative of less total extracellular chloride than was present during the normal control period. However, it has been demonstrated that most of the water lost during the more marked stages of dehydration (above 15 per cent of the body weight) is drawn from the cells. Such a degree of dehydration is accompanied by a loss of body potassium, sodium, and chlorides. The results indicate that the blood serum is first to respond to water deprivation. However, the continued depletion of body potassium evidently means that there is an accompanying loss of cellular water.²³ Furthermore, restoration of the original water content of the body is accompanied by a loss of sodium and a retention of potassium indicating that most of the retained water was being restored to the cells.²

Hematocrit readings, hemoglobin determinations, red blood cell counts, and serum protein determinations in our present studies showed no significant variation, and further suggest that during the degrees of dehydration studied there is no great change in volume of extracellular fluid. Hence the observed increase in the concentration of blood chlorides is an absolute one.

These observations on a normal subject also show that a sharp elevation in concentration of blood urea which resulted from the ingestion of urea is not compensated for by diminution in concentration of plasma chlorides.

At the beginning of the study of the nephritic patient (Table IV) there was a low plasma chloride level, this could have resulted from loss of chlorides by vomiting, associated with low chloride intake, or it might have represented a compensatory reaction for the elevated nonprotein nitrogen. During the first ten days the concentration of the nonprotein nitrogen of the blood fell from 140 mg to an average level of 100 mg per 100 cc and remained at this level. It is significant to note that after the nonprotein nitrogen concentration had reached a relatively stable level, raising the plasma chloride level by feeding sodium chloride did not cause a further fall in the nonprotein nitrogen level.

TABLE III
THIRD STUDY OF NORMAL SUBJECT

DATE	REMARKS	BLOOD UREA NITROGEN MG./100 G.G.	BLOOD PLASMA CHLORIDES AS NaCl MG./100 G.G.	URINE UREA GM.	URINE CHLORIDE AS NaCl GM.	24 HOURLY URINE VOLUME C.C.	OTHER OBSERVATIONS
1/10/37	Period 12: control			14.4	4.2	1830	
1/11/37		9.2	619	15.0	3.5	1400	
1/12/37		8.8	582	16.7	3.4	1680	
1/13/37	Period 13: urea ingestion (20 gm. urea 9:00 A.M., 11:00 A.M., 1:00 P.M.)	9.1	606	42.4	3.9	1640	Hematocrit 49% Hemoglobin 104 (S) R.B.C. 5,680,000 per c.mm. Serum protein Total 8.4 Albumin 4.9 Globulin 3.5
6:00 P.M.		34.4	578				
1/14/37	Period 14: control			24.0	3.1	1660	
1/15/37				16.2	3.2	1120	
6:00 P.M.			573				
1/16/37	Period 15: "hydration"	9.1	578	15.0	5.2	5720	Hematocrit 46.5% Hemoglobin 94 (S) R.B.C. 5,310,000 per c.mm. Serum protein Total 8.5 Albumin 5.1 Globulin 3.4
1/17/37				12.9	3.6	5130	
8:00 A.M.							
6:00 P.M.		7.7	591				

For some days prior to the patient's last readmission, there had been persistent vomiting, and this continued while the patient was under observation. The plasma chloride concentration was 410 mg. per 100 c.c. of blood; this low level can easily be interpreted as due to the emeses.

In the patient with Addison's disease (Table V), as is commonly the case, there was a low plasma chloride level. Were there a reciprocal relationship between the chlorides and the nonprotein nitrogen of the blood, one should expect

TABLE IV
STUDY OF NEPHRITIC PATIENT

DATE	REMARKS	BLOOD NONPROTEIN NITROGEN MG./100 C.C.	BLOOD PLASMA CHLORIDES AS NaCl MG./100 C.C.	AVERAGE DAILY FLUID INTAKE C.C.
(23 days)				
4/21/36		140.0		
5/ 1/36		109.5		
5/ 4/36		106.0	441	
5/ 5/36		105.5	402	
5/ 9/36		93.0	442	
5/11/36		93.0	435	
5/14/36		100.0	438	4087
(15 days)				
5/24/36	7.5 gm. NaCl added to diet			
5/25/36	7.5 gm. NaCl added to diet	121.5	535	
5/26/36	7.5 gm. NaCl added to diet			
5/27/36	7.5 gm. NaCl added to diet			
5/28/36	7.5 gm. NaCl added to diet			
5/29/36	7.5 gm. NaCl added to diet	119.0	565	3844
(5 days)				
6/ 1/36		112.5	580	
6/ 3/36		109.5	575	3477
(6 days)				
7/12/36 to 7/17/36 in- clusive	Readmission	153.0	410	4025

TABLE V
STUDY OF PATIENT WITH ADDISON'S DISEASE

DATE	WHOLE BLOOD CHLORIDE AS NaCl MG./100 C.C.	BLOOD NONPROTEIN NITROGEN MG./100 C.C.
8/ 9/33	453	54.5
8/14/33	457	37.9
8/26/33	415	35.1
9/ 2/33	424	46.0
9/13/33	459	53.1
9/18/33	430	59.4
11/13/33	430	48.0
11/27/33	400	27.8
12/ 4/33	421	40.0
12/18/33	386	41.3
1/ 1/34	424	38.2
1/15/34	386	40.5
2/28/34	430	42.0
3/16/34	412	39.7
4/17/34	379	35.8
5/ 3/34	482	32.9
5/17/34	499	31.5

the latter to be correspondingly elevated in this patient. The data demonstrate that no such relationship exists. For example, the lowest blood chloride value was 379 mg. per cent, and at the same time the blood nonprotein nitrogen value was 35.8 mg. per cent. The highest blood chloride value, 499 mg. per cent, was accompanied by a nonprotein nitrogen value of 31.5 mg. per 100 c.c. of blood. The lowest nonprotein nitrogen value was 27.8 mg. per cent, and the simultaneous blood chloride determination was 400 mg. per cent. The highest nonprotein nitrogen value was 59.4 mg. per 100 c.c. of blood and the simultaneous blood chloride value 430 mg. per 100 c.c.

INTERPRETATION OF PREVIOUSLY PUBLISHED DATA

The observations of Hartmann and Darrow² have been interpreted as evidence that urea is retained not primarily because of the inability of the kidney to excrete it, but in order to compensate for the lowered osmotic pressure resulting from loss of electrolytes. The clinical data presented by these authors indicate that elevation of nitrogenous waste products in the blood is not due to low plasma chlorides, but is associated with either dehydration or very low urinary volume ("urinary suppression").

To illustrate, in one case (No. 20) the patient had vomited repeatedly and urinary output had become very small. Blood chloride concentration was 480 mg. and nonprotein nitrogen concentration 92 mg. per 100 c.c. The urinary output was but 18, 49, 92, 158, and 215 c.c. on successive days, even though the patient was receiving Ringer's solution intraperitoneally, glucose intravenously, and fluids by mouth. Following this, it is stated, the urine volume continued to increase; with this increase there was a fall in nonprotein nitrogen to 40 mg. per cent. Blood chlorides rose to normal.

It is well known that severe dehydration causes marked elevation of nonprotein nitrogen and that it is merely necessary to establish an adequate urinary volume in order to bring the nonprotein nitrogen back to normal.⁴ The data presented here (Table I) are a further example of this. No further explanation for blood nonprotein nitrogen values in these cases need be sought for. It has been repeatedly shown that emesis will cause loss of chlorides from the blood. This is sufficient explanation for the facts in these cases.

Low chloride and high nonprotein nitrogen levels can both be attributed to the vomiting; for these individuals, because of the nausea, do not take fluids by mouth of their own accord and hence frequently enter the hospital in a serious state of dehydration.

In some of the cases presented by Hartmann and Darrow in addition to the vomiting there was fever, which caused a further loss of fluid from the body. The graphic portrayal* of the data in one of the cases presented (No. 24) is extremely interesting inasmuch as the rise in blood urea concentration coincides almost exactly with the period marked "dehydration," and the concentration of the blood chlorides also rose in this period.

*Hartmann and Darrow,² p. 149, Chart 2.

The data included in Hartmann and Darrow's study frequently show plasma chloride and blood nonprotein nitrogen values such as the following (Case No. 25):

	CHLORIDES	NONPROTEIN NITROGEN
7/28/25	604	43.0
8/28/25	491	45.8
10/13/25	624	36.0
9/20/27	614	125.0

Certainly there is no compensatory movement indicated by these two substances.

In the more recent work on this problem¹ the data presented again do not convince us that a reciprocal relationship exists between plasma chlorides and nonprotein nitrogen. It is stated that (in Case 1) the plasma urea nitrogen concentration decreased from 154 to 26 mg. per 100 c.c. of blood during sodium chloride administration. But during this period the patient's intake was "kept rigidly at 4,500 c.c. per day." During the first nineteen days the urinary output was "only slightly above 2.0 c.c. per minute," and the concentration of urea nitrogen fell to 32.8 mg. per 100 c.c. of blood. This daily urinary volume of slightly more than 2,880 c.c. (which soon increased to 3,600 c.c.) was adequate to permit excretion not only of the daily waste solids but also of that which had accumulated in the blood prior to readmission. With a maximal renal concentrating ability such as this patient possessed, 1,010, this volume of urine enabled the kidneys to excrete 90 gm. of solids daily,⁵ which was considerably more than that resulting from the usual daily rate of urea formation. With these data in mind, the fall in urea concentration in the blood can be explained entirely on the provision of an adequate urinary volume.

Later during their study when "salt intake was slightly increased while all other things were kept constant" except the protein intake, which was doubled, the plasma chlorides did not change significantly; however, the concentration of urea nitrogen in the blood rose from 32.8 mg. to 54.5 mg. per cent.

Furthermore, during the four-day period of rigid salt restriction during which the concentration of plasma chlorides fell from 100 to 76 milliequivalents per liter, there was no appreciable alteration in concentration of blood urea nitrogen (38.3 mg. to 41.5 mg. per 100 c.c.), whereas, were there a compensatory mechanism to retain urea in order to maintain osmotic pressure, there should have resulted a large increase in blood urea. These data demonstrate further the lack of any necessary compensatory relationship between plasma chlorides and blood urea.

In Case 2, because there is no appreciable change whatever in the concentration of the plasma chlorides, the feeding and the restriction of sodium chloride cannot have been responsible for the slight changes in blood urea in an effort to maintain the osmotic pressure of the blood.

In Case 3, during the period of salt restriction, it is true that urea nitrogen concentration in the blood rose from 21.7 mg. to 27.7 mg. per cent; and during the period of salt feeding, this value fell to 24.3 mg. per cent. But since plasma

chloride concentration was constant throughout, the movement of blood urea could not be compensatory for shifts in the level of the chlorides in the blood

DISCUSSION

In dealing with this problem, one must consider the state of hydration of the body and the urinary volume in patients with severe impairment of renal function. The facility with which urea is excreted by the kidney is directly related to an adequate supply of water. With a maximal concentrating ability of 1030, the kidney is able to excrete 50 gm of waste solids in as little as 600 cc of urine, but the kidney whose maximal concentrating ability is but 1010 requires a urinary volume of 2,000 cc for the excretion of 50 gm of solid. In patients with such a severe degree of impairment of renal function, only a large urinary volume will allow for the excretion of a sufficient amount of urea, so that its concentration in the blood will decrease. With a fall in the concentration of the nitrogenous waste products in the blood, the nausea and then the vomiting will ordinarily cease. This does away with the extrarenal loss of sodium chloride and also permits the patient to partake of the usual amounts of sodium chloride as part of the diet. It is then noted that the plasma chlorides rise toward the normal.

In our study of a normal subject, a patient with chronic hemorrhagic nephritis, and a patient with Addison's disease there was no evidence of a reciprocal relationship between the nitrogenous waste products and the chlorides in the blood. Furthermore, considering the other data cited, we fail to see the necessity for a compensatory mechanism to explain the changes in concentration of plasma chlorides and nitrogenous waste products in the blood. There is a far simpler explanation for the association of high levels of nitrogenous waste products with low levels of plasma chlorides in the blood. The vomiting depletes the plasma chlorides, and the dehydration concentrates the nitrogenous waste products.

CONCLUSION

It is not necessary to seek a compensatory mechanism to explain the frequent association of hypochloremia and azotemia in chronic nephritis. The quantitative alteration of the blood chemistry is brought about by two conditions common to the disease, namely vomiting and inadequate urinary volume.

Sincere appreciation and gratitude is expressed to Dr L. H. Newburgh and Dr R. H. Freyberg for invaluable guidance and assistance.

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A METHOD OF STUDYING SOME OF THE PHYSIOLOGIC ACTIONS OF BENZEDRINE SULFATE*

REPORT OF TEN CASES

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IN A previous communication we have reported the response of normal persons and depressed patients to the stimulating action of benzedrine sulfate.¹ From the inception of our work with this drug we have been impressed by the physiologic action which we characterized as "variable, uncertain, unpredictable, and at times paradoxical." Therefore, intensive studies are being conducted to accumulate data on the physiologic phenomena. The purpose of this paper is to present the procedure by which such observations are being made, and to make a preliminary report of the protocols of ten typical cases of the series being studied at the Syracuse Psychopathic Hospital. A correlation of the data on a larger group of cases will be undertaken in a future publication.

METHOD OF INVESTIGATION

After several days of careful physical observation, each patient receives daily for ten days to two weeks 10 to 30 mg. of benzedrine sulfate orally, except in instances where untoward effects necessitate withdrawal of the drug. The usual program including nursing care and occupational therapy is in no way modified, except that the fluid intake is limited to approximately 2,000 c.c. each twenty-four hours; no other form of internal medication is administered; and psychotherapy by the physician is reduced to a minimum. Each day during the period of administration observations are made prior to the ingestion of the medication, hourly thereafter for at least five hours, and at additional intervals during the remainder of the twenty-four hours. Data are obtained on the hourly variations of the systolic and diastolic blood pressure, the pulse, the respiration, the temperature, and the vasomotor reactions; on the daily variation in the total urinary output, and the number of stools, and on the alterations in the body weight, the basal metabolic rate, the knee jerks, and the cellular elements of the blood after several days of administration, and at the end of the entire period of study. Investigation of the mental status is conducted as described in the previous communication.¹ This program was devised, because as previously reported, the reactions became apparent from one to three hours after the administration of the drug, and persisted from three to eight or nine hours; and because the data thus obtained might be more easily applied clinically.

*From the Syracuse Psychopathic Hospital and the Department of Psychiatry of the Syracuse University College of Medicine, Dr. Harry A. Steckel, Director.
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The following protocols make the method of procedure obvious:

CASE 1.—NORMAL

OBSERVATION INTERVAL	TIME AFTER MEDICATION IN HOURS	BLOOD PRESSURE	PULSE	TOTAL DAILY URINARY OUTPUT	NUMBER OF BOWEL MOVEMENTS	BODY WEIGHT IN POUNDS	ADDITIONAL OBSERVATIONS
4/13/37	-	125/75	100	-	1	147	
4/18/37	-	123/80	98	760	1	147	
4/19/37	-1	120/78	110	780	0	-	<i>Alterations in Mental Status:</i>
First day of medication 20 mg.	0	114/76	100				Increased motor activity; slight elevation in mood; irritability.
	1	124/70	108				
	2	126/76	102				
	3	128/78	100				<i>Skin and Mucous Membranes and Vasomotor System:</i>
	4	130/68	104				Impending syncope, blurring vision, abdominal cramps, circumoral pallor, perspiration, dryness of mouth, headache, flushing of face, nausea, chills
	5	128/70	100				
	6	130/66	100				
	7	124/68	96				
	14	-	102				
	18	-	118				
4/20/37	-2	115/75	110	900	1	146	<i>Temperature:</i> Daily fall of 1 to 2 degrees 12 hours after administration
Second day of medication 20 mg	0	128/68	100				
	2	130/70	110				
	3	130/76	88				<i>Basal Metabolic Rate (Du Bois):</i> 4/18/37 minus 7 per cent; 4/23/37 minus 4 per cent.
	7	138/84	112				
	12	-	106				
4/22/37	0	120/68	108	1140*	0	145	<i>Knee Jerks:</i> Increase from plus 2 to plus 4
Fourth day of medication 20 mg.	1	132/72	112				
	2	148/80	92				<i>Cellular Elements in the Blood.</i>
	3	130/86	88				
	7	130/70	88				
	8	140/80	96				
	16	-	118				
4/23/37	10	134/04	110	-	0	145	
First day after medication	A.M.						Hgb. 4/18/37 85% 4/23/37 90%
	3	118/72	102				RBC† 4,890 5,030
	P.M.						WBC. 9,850 7,100
4/25/37	10	116/70	100	-	-	-	Poly. 70% 50%
Third day after medication	A.M.						Lymph. 25% 43%
	4	126/70	96				L. Mono 1% 3%
	P.M.						Eos. 4% 3%
							Bas. 0 1%
4/30/37	-	125/70	88	-	-	146	<i>Respirations:</i> Variation from 18 to 24.
Eighth day after medication							

*Fluid intake increased 300 cc because of excessive thirst.

†Last three figures omitted

RÉSUMÉ OF PHYSIOLOGIC DATA

1. *Blood Pressure.*—Unpredictable increase or decrease in both the systolic and the diastolic levels occurring together or independently. Attention is directed to the diastolic variations, as in Cases 3, 4, 6, and 9. Increased levels are more frequent.

2. *Pulse.*—Increase or decrease in rate, not necessarily concomitant with blood pressure variations, as in Cases 2 and 3; arrhythmias occasionally observed, as in Case 7.

CASE 2.—INVOLUTION MELANCHOLIA

OBSERVATION INTERVAL	TIME AFTER MEDICATION IN HOURS	BLOOD PRESSURE	PULSE	TOTAL DAILY URINARY OUTPUT*	NUMBER OF BOWEL MOVEMENTS	BODY WEIGHT IN POUNDS	ADDITIONAL OBSERVATIONS
2/10/37	-	146/100	74	-	0	147	<i>Alterations in Mental Status:</i> No elevation of mood, increase in speech and motor activity, irritability.
2/24/37	0	130/90	84	1000	2	145	
Day prior to medication	7	130/90	104				
2/25/37	0	126/90	92	1645	0	-	<i>Skin and Mucous Membranes and Vasomotor System:</i> Perspiration, pains in legs, abdominal cramps, chills, headache.
First day of medication 20 mg.	3	132/92	140				
	5	140/94	128				
	7	132/90	96				<i>Temperature:</i> Vespertal increase 1.5 degrees for first two days, 0.5 degrees on most days thereafter.
2/26/37	0	134/98	114	470	2	145	
Second day of medication 20 mg.	1	126/90	96				
	5	140/94	140				<i>Basal Metabolic Rate (Du-Bois):</i> 2/24/37 minus 10 per cent; 2/26/37 plus 5 per cent; 3/2/37 plus 1 per cent.
	8	134/92	128				
2/27/37	0	118/80	94	680	2	-	
Third day of medication 20 mg.	5	124/92	118				<i>Knee Jerks:</i> Unchanged.
2/28/37	0	116/92	120	1125	0	-	
Fourth day of medication 20 mg.	4	140/90	130				<i>Cellular Elements of the Blood:</i> 2/24/37 2/26/37 3/2/37 Hgb. 85% 85% 90% R.B.C.† 4,460 4,970 5,170 W.B.C. 6,800 7,550 7,350 Poly. 52% 62% 52% Lymph. 42% 31% 44% L. Mono. 4% 7% 4% Eos. 2% 0 0 Bas. 0 0 0
	5	128/90	140				
	9	112/92	120				
3/1/37	0	124/90	96	475	1	-	<i>Respirations:</i> Variation from 18 to 22.
Fifth day of medication 20 mg.	2	128/88	120				
	8	118/88	96				
3/2/37	0	110/88	106	1025	enema	145	
Sixth day of medication 20 mg.	2	116/84	110				
	8	134/86	96				
3/3/37	0	128/92	90	1050	0	-	
Seventh day of medication 20 mg.	4	134/92	128				
	8	132/88	96				
3/4/37	0	114/92	100	900	enema	-	
Eighth day of medication 20 mg.	1	134/100	142				
	9	120/90	96				
3/6/37	0	112/80	90	600	0	-	
Tenth day of medication 20 mg.	5	128/86	104				
	8	120/86	96				
3/8/37	0	118/80	80	500	enema	-	
Twelfth day of medication 20 mg.	5	112/84	114				
	8	114/84	96				
3/9/37	0	106/80	98	625	0	-	
Thirteenth day of medication 20 mg.	4	126/88	116				
	8	126/84	92				
3/11/37	0	112/76	120	800	0	-	
Fifteenth day of medication 20 mg.	1	102/76	100				
	5	116/86	118				
	8	108/74	96				
3/13/37	0	108/72	92	1025	1	-	
Seventeenth day of medication 20 mg.	3	122/82	112				
	5	112/82	120				
3/14/37	0	104/72	98	-	0	144	
Eighteenth day of medication 20 mg.	3	118/90	108				

*Urinary output for 12 hours only.

†Last three figures omitted.

CASE 3—PSYCHOSIS WITH POSTENCEPHALITIC PARKINSONISM

OBSERVATION INTERVAL	TIME AFTER MEDI- CATION IN HOURS	BLOOD PRESSURE	PULSE	TOTAL DAILY URINARY OUTPUT	NUMBER OF BOWEL MOVEMENTS	BODY WEIGHT IN POUNDS	ADDITIONAL OBSERVATIONS
5/1/37	-	114/90	80	-	0	152	<i>Alterations in Mental Status</i> Elevation of mood increase in speech and motor activity, irritability and fatigue, transitory confusion on seventh day
5/11/37	-	120/70	84	1440	1	154	
5/13/37	0	118/68	94	2065	2	-	
First day of medication 20 mg	4	120/68	112				
	6	124/78	120				
	7	120/80	100				<i>Skin and Mucous Membranes and Vasomotor System</i> Flushed face, sweating of the hands, greyness of the skin, dizziness, dryness of the mouth injection of conjunctivae, increase in nasal secretion palpitation and precordial pain on seventeenth day
5/13/37	0	108/72	100	1555	2	-	
Second day of medication 20 mg	4	126/70	124				
	8	128/90	96				
5/14/37	0	110/68	84	1525	1	-	
Third day of medication 20 mg	4	130/94	100				<i>Temperature</i> On most days a vesperal rise of 2 degrees over the average minimal level of 97 degrees. Pyrexmistrative variations up to 2 degrees noted
	6	120/78	110				
	9	118/75	78				
5/15/37	0	108/70	80	2640	3	-	
Fourth day of medication 20 mg	1	120/76	84				
	7	90/60	92				
5/16/37	-1	110/70	72	1040	2	-	<i>Basal Metabolic Rate (Du Bois)</i> 5/11/37 minus 6 per cent 5/17/37 plus 17 per cent 5/20/37 plus 2 per cent
Fifth day of medication 20 mg	4	128/80	100				
	8	110/70	88				
	9	126/80	102				
5/17/37	0	108/60	80	1845	2	156	
Sixth day of medication 20 mg	2	120/76	100				<i>Knee Jerks</i> Unchanged <i>Cellular Elements of the Blood</i> 5/11/37 5/17/37 5/20/37 Hgb 100% 95% 95% RBC * 6,000 5,030 5,870 WBC 13,900 10,450 11,700 Poly 50% 46% 58% Lymph 43% 49% 30% L Mono 4% 4% 4% Eos 3% 1% 2% Bas 0 0 0
	6	128/88	114				
	8	104/68	118				
5/18/37	0	104/60	90	625	2	-	
Seventh day of medication 20 mg	2	112/76	100				
	7	130/86	86				
5/19/37	0	112/76	80	1495	2	-	<i>Respirations</i> Variation from 18 to 20
Eighth day of medication 20 mg	3	112/80	104				
	7	130/86	90				
5/20/37	1	112/76	80	800	2	152	
Ninth day of medication 20 mg	4	114/88	88				
	8	126/78	96				
5/21/37	0	112/80	94		1	-	
Tenth day of medication 20 mg	6	118/80	80				
5/24/37	0	98/78	88		-	-	
Twenty fourth day of medication 15 mg							

*Last three figures omitted

3 *Bowel and Urinary Functions*—Increase or decrease in frequency of action, increase or decrease in amount of excretion

4 *Body Weight*—Decrease in most subjects, occasional marked increase following discontinuance of the drug, as in Cases 6 and 7

5 *Skin, Mucous Membranes, and Vasomotor System*—Noted in detail in the protocols and described in the previous communication¹ Attention is again

CASE 4.—PSYCHONEUROSIS, NEURASTHENIA (ACCOMPANIED BY HYPERTENSION AND EARLY ARTERIOSCLEROSIS)

OBSERVATION INTERVAL	TIME AFTER MEDICATION IN HOURS	BLOOD PRESSURE	PULSE	TOTAL DAILY URINARY OUTPUT	NUMBER OF BOWEL MOVEMENTS	BODY WEIGHT IN POUNDS	ADDITIONAL OBSERVATIONS
9/23/36	-	194/122	56	-	1	86	<i>Alterations in Mental Status:</i> Increase in talkativeness, slight increase in motor activity, marked irritability, increase in depression.
11/17/36 Two weeks prior to medication	-	140/90	70	-	2	92	
11/30/36 Day prior to medication	-	136/84	76	1500	1	95	<i>Skin and Mucous Membranes and Vasomotor System:</i> Medication discontinued because of headache, dizziness, syncope, blurring vision, staggering, accompanying the rise in the systolic and diastolic blood pressure levels.
12/1/36 First day of medication 20 mg.	0	140/90	76	2000	0	-	
	1	224/122					
	2	208/132	82				
	3	160/118					
	4	144/98					
	7	140/96					<i>Temperature:</i> Variation from 98.0 to 98.6 degrees.
12/2/36 Second day of medication 10 mg.	0	142/92	72	1050	2	-	
	1	210/120					<i>Basal Metabolic Rate (DuBois):</i> 10/1/36 minus 12 per cent; 11/30/36 plus 3 per cent; 12/2/36 minus 6 per cent.
	2	165/105					
	3	158/90	80				
	5	138/80					
	6	128/86	78				
	10	136/78					<i>Knee Jerks:</i> Decrease from plus 2 to plus 1.
12/3/36 First day after medication (placebo)	0	145/95	76	1350	0	92	
	1	150/100					<i>Cellular Elements of the Blood:</i> 11/30/36 12/2/36 Hgb. 80% 85% R.B.C.* 4,810 4,650 W.B.C. 5,750 5,500 Poly. 73% 64% Lymph. 23% 28% L. Mono. 2% 8% Eos. 1% 0 Bas. 1% 0
	2	158/88					
	3	150/80					
	8	122/82					
	10	138/86					
12/4/36 Second day after medication (no medication)	-	159/102	72	-	2	-	
1/18/37 Forty-seven days after medication	-	174/114	62 84	-	-	-	<i>Respirations:</i> Variation from 18 to 22.

*Last three figures omitted.

directed to the untoward effects, particularly impending collapse, as in Cases 1 and 7.

6. *Other Functions.*—Temperature—slight increase or decrease; basal metabolic rate—increase more often than decrease but no consistent variation; knee jerks—occasional increase; cellular elements of the blood—no striking variation, except for a moderate increase in Case 5; respiration—relatively unaffected.

COMMENT

No attempt is made at this time to correlate the data presented in the foregoing summary. The results appear variable and uncertain, but sufficiently striking at times to justify the intensive study the procedure entails.

CASE 5—TRAUMATIC PSYCHOSIS, POSTTRAUMATIC PERSONALITY DISORDER

OBSERVATION INTERVAL	TIME AFTER MEDI- CATION IN HOURS	BLOOD PRESSURE	PULSE	TOTAL DAILY URINARY OUTPUT	NUMBER OF BOWEL MOVEMENTS	BODY WEIGHT IN POUNDS	ADDITIONAL OBSERVATIONS																																													
2/18/37	-	130/70	84	-	-	127	Alterations in Mental Status Some elevation of mood, increase in irritability, increase in speech, motor activity, and efficiency, IQ raised from 68 to 79																																													
2/24/37	-	120/75	80	870	0	132																																														
2/25/37	0	120/75	80	1080	0	-																																														
First day of medication 20 mg	2	114/66	76				Skin and Mucous Membranes and Vasomotor System Marked flush of face, increase in nasal discharge, parching of mouth, increase in perspiration, headache																																													
	3	124/64	84																																																	
	4	108/68	96																																																	
	10	115/70	80																																																	
2/26/37	0	110/66	80	1975	3	140	Temperature Practically constant at 99.0 degrees																																													
Second day of medication 20 mg	1	116/70	96																																																	
	2	118/76	80																																																	
	3	120/80	88																																																	
2/27/37	0	108/78	80	1700	3	-	Basal Metabolic Rate (DuBois) 2/24/37 plus 11 per cent, 2/26/37 zero per cent, 3/3/37 plus 20 per cent, 3/9/37 plus 7 per cent																																													
Third day of medication 20 mg	2	100/68	82																																																	
	5	112/68	80																																																	
2/28/37	0	100/76	70	1745	1	-	Knee Jerks Increase from plus 3 to plus 4																																													
Fourth day of medication 20 mg	7	124/80	68																																																	
	8	122/76	66																																																	
	13	116/80	70																																																	
3/3/37	0	110/68	80	1900	0	130	Cellular Elements of the Blood																																													
Seventh day of medication 20 mg	8	124/86	80																																																	
	13	106/76	76																																																	
3/4/37	0	112/70	70	1150	enema		<table><tr><td></td><td>2/24/37</td><td>2/26/37</td><td>3/3/37</td><td>3/9/37</td></tr><tr><td>Hgb</td><td>95%</td><td>95%</td><td>95%</td><td>100%</td></tr><tr><td>RBC*</td><td>5,230</td><td>5,090</td><td>4,950</td><td>6,070</td></tr><tr><td>WBC</td><td>11,350</td><td>11,200</td><td>9,350</td><td>12,600</td></tr><tr><td>Poly</td><td>58%</td><td>63%</td><td>63%</td><td>72%</td></tr><tr><td>Lymph</td><td>37%</td><td>29%</td><td>28%</td><td>20%</td></tr><tr><td>L Mono</td><td>5%</td><td>2%</td><td>8%</td><td>4%</td></tr><tr><td>Eos</td><td>0</td><td>0</td><td>1%</td><td>3%</td></tr><tr><td>Bas</td><td>0</td><td>0</td><td>0</td><td>1%</td></tr></table>		2/24/37	2/26/37	3/3/37	3/9/37	Hgb	95%	95%	95%	100%	RBC*	5,230	5,090	4,950	6,070	WBC	11,350	11,200	9,350	12,600	Poly	58%	63%	63%	72%	Lymph	37%	29%	28%	20%	L Mono	5%	2%	8%	4%	Eos	0	0	1%	3%	Bas	0	0	0	1%
	2/24/37	2/26/37	3/3/37	3/9/37																																																
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	2/24/37	2/26/37	3/3/37	3/9/37																																																
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Eos	0	0	1%	3%																																																
Bas	0	0	0	1%																																																
Ninth day of medication 20 mg	0	138/78	80																																																	
	12	102/64	92																																																	
3/6/37	0	112/64	68	1400	enema	-	Cells A few young polys noted 3/9/37.																																													
Tenth day of medication 30 mg.	3	120/74	80																																																	
	5	126/80	68																																																	
	12	118/80	80																																																	
3/7/37	0	112/66	84	2000	0	-	Respirations Variation from 18 to 20																																													
Eleventh day of medication 30 mg	4	124/80	86																																																	
	7	130/90	78																																																	
	12	134/70																																																		
3/8/37	0	116/70	70	1200	enema	-																																														
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	4	138/90	88																																																	
	5	136/85	90																																																	
	9	138/78	80																																																	
3/10/37	-	120/76	80	-	-	-																																														
First day after medication	-																																																			
3/23/37	-	116/76	88	-	-	-																																														
Fourteenth day after medication	-																																																			

*Last three figures omitted

CASE 6.—PSYCHOSIS WITH MENTAL DEFICIENCY, EPISODE OF EXCITEMENT FOLLOWED BY DEPRESSION

OBSERVATION INTERVAL	TIME AFTER MEDICATION IN HOURS	BLOOD PRESSURE	PULSE	TOTAL DAILY URINARY OUTPUT	NUMBER OF BOWEL MOVEMENTS	BODY WEIGHT IN POUNDS	ADDITIONAL OBSERVATIONS
1/7/37	-	120/45	68 56	-	-	128	<i>Alterations in Mental Status:</i> Increase in irritability, motor activity and speech activity; no change in I. Q.
1/13/37	-	108/48	68	-	1	128	
1/14/37	0	108/48	68	1860	0	-	
First day of medication 20 mg.	1	96/40	64				<i>Skin and Mucous Membranes and Vasomotor System:</i> Coldness of the extremities.
	3	102/40	72				
	5	112/54					
	7	124/70	80				
1/15/37	-1	110/50	66	1640	1	-	<i>Temperature:</i> Practically constant at 99.0 degrees. <i>Basal Metabolic Rate (Du Bois):</i> 1/13/37 minus 5 per cent; 1/21/37 plus 2 per cent; 1/25/37 minus 2 per cent.
Second day of medication 20 mg.	2	120/60					
	5	130/80					
	6	126/72					
1/16/37	0	112/50	68	850	0	-	<i>Knee Jerks:</i> Unchanged. <i>Cellular Elements of the Blood:</i> 1/13/37 1/21/37 1/25/37 Hgb. 95% 100% 100% R.B.C.* 4,670 5,180 5,130 W.B.C. 6,150 6,700 7,050 Poly. 54% 50% 71% Lymph. 39% 40% 23% L. Mono. 6% 8% 6% Eos. 0 2% 0 Bas. 1% 0 0
Third day of medication 20 mg.	5	118/74	80				
	7	120/68	68				
	8	126/68	76				
1/17/37	1	108/40	70	1310	1	-	<i>Respirations:</i> Variation from 16 to 20.
Fourth day of medication 20 mg.	3	112/40	76				
	4	110/38	60				
	5	118/40	68				
1/18/37	0	112/70	68	850	0	-	
Fifth day of medication 20 mg.	5	114/74	80				
	0	110/40	70	900	1	-	
	4	110/60	80				
1/19/37	0	110/40	70	900	1	-	
Sixth day of medication 20 mg.	4	110/60	80				
	5	114/70	72				
	8	126/84	84				
1/20/37	0	114/68	68	1250	1	-	
Seventh day of medication 20 mg.	6	130/84	80				
	7	126/78	86				
	0	120/70	70	850	0	130	
1/21/37	0	120/70	70	850	0	130	
Eighth day of medication 20 mg.	6	128/84	88				
	8	126/80					
	0	110/70	70	1500	1	-	
1/22/37	0	110/70	70	1500	1	-	
Ninth day of medication 20 mg.	4	120/76	66				
	8	118/70	82				
	0	108/60	60	1300	1	-	
1/23/37	0	108/60	60	1300	1	-	
Tenth day of medication 20 mg.	3	120/70	80				
	4	128/68	74				
	8	120/80	80				
1/24/37	0	120/68	70	1250	0	-	
Eleventh day of medication 20 mg.	3	124/68	66				
	4	130/76	88				
	8	120/68	76				
1/25/37	2	120/68	84	800	1	131+	
Last day of medication 20 mg.							

*Last three figures omitted. †Weight 141 pounds sixteen days after medication.

CASE 7—PSYCHONEUROSIS, ANXIETY Hysteria

OBSERVATION INTERVAL	TIME AFTER MEDICA- TION IN HOURS	BLOOD PRESSURE	PULSE	TOTAL DAILY URINARY OUTPUT	NUMBER OF BOWEL MOVEMENTS	BODY WEIGHT IN POUNDS	ADDITIONAL OBSERVATIONS
12/29/36	-	148/86	85	-	0	120	<i>Alterations in Mental Status</i> Some elevation in mood, in- crease in speech and motor activity, irritability and fa- tigue, transitory delirium with tactile hallucinations
1/10/37	-	108/60	80	700	1	124	
1/11/37	0	108/60	86	1000	0	-	
First day of med- ication 20 mg	2 3 4 7	128/90 130/88 138/90 122/68	92 84 78				
1/12/37	0	112/68	56	1470	0	-	<i>Skin and Mucous Membranes and Vasomotor System</i> Ar- rhythmia, flushing of face, perspiration, parching of mouth, dizziness, blurring of vision, cyanosis of lips, cir- cumoral pallor, headache
Second day of medication 20 mg	4 6 7 8	136/82 126/68 134/80 130/68	100 104 88 84				
1/13/37	0	118/68	68	1200	enema	123	
Third day of med- ication 20 mg	5 8 9	140/84 128/70 134/80	66 66 68				
1/14/37	0	112/76	68	1800	0		<i>Temperature</i> Variation from 99.0 to 99.4 degrees <i>Basal Metabolic Rate (Du Bois)</i> 1/10/37 minus 1 per cent, 1/13/37 minus 6 per cent, 1/18/37 minus 11 per cent <i>Knee Jerks</i> Increase from plus 2 to plus 4 <i>Cellular Elements of the Blood</i> 1/10/37 1/18/37 Hgb 100% 90% RBC* 5,100 4,620 WBC 7,050 5,950 Poly 47% 48% Lymph 45% 41% L Mono 4% 7% Eos 2% 3% Bas 2% 1% <i>Respirations</i> Variation from 16 to 22
Fourth day of medication 20 mg	3 4	128/78 140/90	80 70				
1/15/37	-1	120/72	88	1850	enema		
Fifth day of med- ication 20 mg	2 3 4 6 8	150/70 140/80 130/70 130/70 126/80	68 70 80 112 84				
1/16/37	0	128/80	82	1280	0		<i>Respirations</i> Variation from 16 to 22
Sixth day of med- ication 10 mg	2 4 6	124/88 124/80 120/82	90				
1/17/37	0	112/70	80	1550	enema	-	
Seventh day of medication 10 mg	8	120/88	90				
1/18/37	0	112/68	64		1	122	<i>Respirations</i> Variation from 16 to 22
Eighth day of medication 10 mg	2	124/72	68				
1/19/37	0	128/72		-	-		
First day after medication							
2/4/37	0	140/84	74	-	-	128	
Seventeenth day after medication							

*Last three figures omitted

It is recognized that there is variability in the action of both the sympathetic and the parasympathetic drugs. Benzedrine sulfate, which is classed with the former, appears to be no exception to this. Its unpredictable action suggests that the response of each individual to the drug tends to vary with each individual as a total unit depending on the interaction of a number of

OBSERVATION INTERVAL	TIME AFTER MED- ICATION IN HOURS	BLOOD PRESSURE	PULSE	TOTAL DAILY URINARY OUTPUT	NUMBER OF BOWEL MOVEMENTS	BODY WEIGHT IN POUNDS	ADDITIONAL OBSERVATIONS
11/16/36	-	124/76	76	-	-	107	<i>Alterations in Mental Status:</i> Elevation of mood, increase in speech activity, motor ac- tivity, fatigue at times.
11/24/36	-	106/62	60	-	1	114	
11/25/36	0	112/60	60	810	0	-	
First day of med- ication 20 mg.	1	100/58	66				<i>Skin and Mucous Membranes and Vasomotor System:</i> Se- vere headache.
	2	96/50					
	3	100/54	68				
	5	96/58	70				
	6	116/92	70				
11/26/36	0	106/60	66	625	0	-	<i>Temperature:</i> Variation from 98.0 to 99.0 degrees.
Second day of medication 20 mg.	2	106/60					
	3	108/70					<i>Basal Metabolic Rate (Du- Bois):</i> 11/24/36 minus 13 per cent; 12/1/36 plus 28 per cent; 12/8/36 plus 3 per cent.
	4	110/60	68				
	7	118/80	68				
11/27/36	0	112/68	68	1375	0	-	<i>Knee Jerks:</i> Unchanged.
Third day of med- ication 20 mg.	3	124/80					
	6	108/64					
	7	98/70	68				<i>Cellular Elements of the Blood:</i> 11/24/36 12/8/36 Hgb. 95% 95% R.B.C.* 5,580 5,220 W.B.C. 7,750 10,200 Poly. 66% 65% Lymph. 24% 19% L. Mono. 3% 11% Eos. 5% 3% Bas. 2% 2%
11/28/36	0	108/70	64	1000	enema	-	
Fourth day of medication 20 mg.	2	98/94	66				
	4	114/76					
	5	104/68	68				
11/29/36	0	100/70	68	850	1	-	<i>Respirations:</i> Variation from 16 to 20.
Fifth day of med- ication 20 mg.	4	108/80					
	7	110/86	68				
11/30/36	0	118/68	68	900	0	-	
Sixth day of med- ication 20 mg.	1	112/66					
	2	130/76					
	6	112/66					
	7	118/76	70				
12/3/36	0	118/70	68	1200	0	-	
Ninth day of med- ication 30 mg.	5	126/70					
	9	120/90	70				
12/5/36	0	122/86	66	1125	1	-	
Eleventh day of medication 10 mg. at 0 hr. 20 mg. at 6 hr.	4	118/68					
	9	128/72	66				
12/6/36	0	126/70	68	850	0	-	
Twelfth day of medication 10 mg. at 0 hr. 20 mg. at 8 hr.	2	124/65					
	7	104/72					
	12	124/74					
	18	116/70	66				
12/7/36	0	118/68	68	1370	enema	-	
Last day of med- ication 10 mg. at 0 hr. 20 mg. at 8 hr.	3	130/72					
	5	124/68					
	7	110/68					
	12	120/68	68				
12/8/36	-	130/72	68	-	-	111	
First day after medication							
12/10/36	-	118/68	-	-	-	-	
Third day after medication							
12/14/36	-	120/80	84	-	-	-	
One week after medication							
12/21/36	-	120/68	-	-	-	-	
Two weeks after medication							

*Last three figures omitted.

CASE 9 — ALCOHOLIC PSYCHOSIS, ACUTE HALLUCINOSIS, FOLLOWED BY DEPRESSION

OBSERVATION INTERVAL	TIME AFTER MEDI- CATION IN HOURS	BLOOD PRESSURE	PULSE	TOTAL DAILY URINARY OUTPUT	NUMBER OF BOWEL MOVEMENTS	BODY WEIGHT IN POUNDS	ADDITIONAL OBSERVATIONS
10/21/36	-	132/96	78		0	189	Alterations in Mental Status
11/4/36		112/78 100/70	120	950	1	184	Elevation of mood, increase in motor activity, increase in irritability, increase in abili- ty to concentrate, marked fearfulness
11/5/36	0	116/78	110	450	2		Skin and Mucous Membranes and Vasomotor System Free flushed, perspiration, numb- ness of hands and feet, gen- eralized tremors
First day of med- ication 20 mg	1	130/98					
	2	158/88					
	3	138/100					
	6	130/90	90				
11/6/36	-1	110/90	92	750	2	-	Temperature Variation from 98.8 to 99.8 degrees
Second day of medication 20 mg	1	104/68					
	2	124/84					
	6	122/94	80				
11/7/36	0	125/88	78	550	3	183	Basal Metabolic Rate (Du- Bois) 11/4/36 minus 2 per cent, 11/7/36 plus 1 per cent 11/10/36 plus 17 per cent
Third day of med- ication 20 mg	2	140/94					
	3	98/60					
	6	122/94					
	9	134/104	120				
11/8/36	1	102/78	70	1200	2	-	Knee Jerks 11/4/36 absent, 11/10/36 plus 3
Fourth day of medication 20 mg	1	138/98					
	3	142/92	78				
11/9/36	-5	138/84	74	950	2		Cellular Elements of the Blood 11/4/36 11/7/36 11/10/36
Fifth day of med- ication 30 mg	-2	134/96					
	-1	130/88					
	2	130/88					
	6	126/90	74				
11/10/36	-1	110/90	72		2	179	Hgb 85% - 90%
Sixth day of med- ication 30 mg	1	150/100					RBC * 4,530 - 4,710
	2	158/104					WBC 6,350 6,500 6,200
	4	152/102					Poly 76% 67% 66%
	6	128/98					Lymph 16% 25% 33%
11/11/36	0	102/70	70	-			I Mono 4% 6% 0
First day after benzedrine (placebo)	6	98/68					Eos 2% 2% 1%
							Ba ⁴ 2% 0 0
11/12/36	0	110/78			1	-	Cells A few young polys noted 11/10/36
Second day after benzedrine (placebo)	6	124/74					Respirations Variation from 18 to 20

*Last three figures omitted

factors rather than on the peripheral action alone. Among these factors might be included the physical constitution of the individual, his endocrine status, his idiosyncrasy, his personality makeup and central affectual tendency, his psychologic reaction to the procedure based on his previous conditioning, existing mechanisms and complex formations, the effect of this psychologic state on the physiologic reaction, the additional physiologic conditioning resulting from the procedure, the personality of the investigator and the reaction of the patient to him, the state of the entire nervous system, including the voluntary, the reflex, and possibly the extrapyramidal systems and particularly the state

CASE 10.—DEMENTIA PRAECOX, CATATONIC TYPE

OBSERVATION INTERVAL	TIME AFTER MED- ICATION IN HOURS	BLOOD PRESSURE	PULSE	TOTAL DAILY URINARY OUTPUT	NUMBER OF BOWEL MOVEMENTS	BODY WEIGHT IN POUNDS	ADDITIONAL OBSERVATIONS
12/8/36	-	128/82	84	-	-	160	<i>Alterations in Mental Status:</i> Increase in motor activity.
12/13/36	-	110/64	86	2200	0	163	
12/14/36	0	110/62	84	890	0	-	<i>Skin and Mucous Membranes and Vasomotor System:</i> Un- changed.
First day of med- ication 20 mg.	2	120/80					
	5	138/74	76				
	7	138/80					
12/15/36	0	138/80	74	1050	1	-	<i>Temperature:</i> Variation from 99.0 to 99.4 degrees.
Second day of medication 20 mg.	2	140/80					
	4	140/84	72				
	8	132/76	72				
12/16/36	0	126/80	70	1100	0	-	<i>Basal Metabolic Rate (Du- Bois):</i> 12/13/36 minus 9 per cent; 12/22/36 plus 13 per cent.
Third day of med- ication 20 mg.	4	140/82	88				
	6	136/74	88				
12/18/36	0	130/80	86	740	enema	-	<i>Knee Jerks:</i> Unchanged.
Fifth day of med- ication 20 mg.	5	138/80					
	6	136/84	80				
	8	130/86					
12/19/36	0	126/82	80	1000	0	-	<i>Cellular Elements of the Blood:</i> 12/13/36 12/22/36 Hgb. 100% 100% R.B.C.* 5,250 5,070 W.B.C. 8,500 5,050 Poly. 62% 69% Lymph. 24% 24% L. Mono. 10% 2% Eos. 2% 3% Bas. 2% 2%
Sixth day of med- ication 20 mg.	3	140/76					
	6	134/78	70				
	7	136/90					
	8	138/78					
12/21/36	0	126/78	86	800	ca- thar- tic	-	
Eighth day of medication 20 mg.	4	140/80					
	9	138/76	70				
12/22/36	-	126/74	88	-	-	160	<i>Respirations:</i> Variation from 16 to 20.
First day after medication							

*Last three figures omitted.

of balance and tonus of the components of the entire vegetative nervous system.³ The reaction of the autonomic nervous system may involve the state of the so-called "sympathetic centers" and the parasympathetic centers in the cortical (Orlandie), the dienecephalic (hypothalamie), the mesencephalic, and the medullary-spinal regions; the state of the sympathetic and parasympathetic ganglia; the state of the sympathetic and parasympathetic efferent nerve fibers and their endings; and the tendency to specific circumscribed reactions, compensatory reactions, delayed responses, and aftereffects as pointed out by Pavlov⁴ in his statements on positive and negative induction, and Sherrington⁵ in his description of immediate and successive induction as part of the integrative action of the central nervous system.

SUMMARY

1. A method of procedure is described by which some of the physiologic actions of benzedrine sulfate are being studied clinically, particularly in regard

to the effect on the blood pressure, the pulse, the bowel and the urinary functions, the body weight, the skin, the mucous membranes, and the vasomotor system. Observations are also being recorded on the variations in temperature, the basal metabolic rate, the cellular elements of the blood, the knee jerks, and the respiration.

2. Ten typical protocols are presented to illustrate the type of data obtained by this procedure. The results, variable and uncertain as they appear to be, are sufficiently interesting to warrant continued careful study.

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CHOLESTEROLYSIS IN THE BLOOD PLASMA OF NORMAL MAN

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THE observation of Shope¹ that cholesterol ester may disappear entirely from the blood serum of animals after death led him to postulate the presence of some very active cholesterol ester splitting substance in animal tissues. He then incubated the blood serum of a cow with cholesterol free, saline extracts of different tissues, and in each instance obtained a more or less marked hydrolysis of cholesterol esters. The proportion of free to combined cholesterol in the serum was not altered. Sperry² in opposition to this, examined the postmortem blood of 150 persons who had died suddenly from various causes, usually trauma. He found that the percentage of free in total cholesterol was within the normal range (24 to 30 per cent) in 115 instances, below 24 per cent in 14 instances, and above 30 per cent in 21 instances. Sperry² has further declared that an active cholesterol esterase is present in blood serum and that a definite esterification of free cholesterol takes place when the blood serum has been incubated, with a resulting large increase in the proportion of combined to free cholesterol. Kondo,³ Cytionberg,⁴ Mueller,⁵ Schultz,⁶ and Thannhauser⁷ had incubated whole blood and obtained the opposite results, i.e., that there was either a hydrolysis of the

cholesterol esters or no significant change in the proportion of combined to free cholesterol. Schultz⁶ has incubated mixtures of blood serum or plasma with minced liver and noted no change in the cholesterol fractions, although a marked splitting of cholesterol esters did occur when minced liver was incubated with whole blood. Mueller⁵ and Nomura⁸ have noticed the esterification of free cholesterol when they incubated blood serum with pancreas and pancreas extracts. Opposed to this are the results of the work of Kondo,³ Schultz,⁶ Cytronberg,⁴ Thannhauser,⁷ and Nomura⁹ who reported that cholesterol esters are hydrolyzed when they are incubated with different tissues, including the body fluids, pancreas, and tissue extracts. Sperry,¹¹ using saline extracts of liver, kidney, muscle, lung, brain, spleen, and heart of the guinea pig, mouse, and rat and incubating them with beef, sheep, and human blood serum found that an esterification of free cholesterol occurs just as when serum was incubated alone, but that in most instances the tissue extracts appeared to inhibit the reaction to some extent. Sperry,² incubating blood serum at 37 to 40° C. for as long as three days in some instances, noticed that in most cases the total cholesterol before and after incubation agreed within the limit of error of the procedure. In a few instances the differences were slightly greater, but they varied equally in each direction.

Although the above investigations may appear conflicting and confusing, it would seem that there is definite proof in them that incubation of blood serum or whole blood results in the activation of a cholesterol esterase, with a resulting conversion of free cholesterol to the combined form. In the incubated samples there would result, as Sperry has indicated and providing there was no change in the total cholesterol, an increase in the proportion of the combined to the free forms. This resulting total cholesterol, with the exception of Sperry's² work, has been neglected and has not been too carefully investigated. The problem of the rate of change, if any, taking place in the total cholesterol of blood over a time period should be established, because knowledge of this type is important for practical as well as theoretical reasons. It is the purpose of this paper to present such an investigation.

METHOD

The individuals used for this work were normal both physically and mentally. All were males, females being excluded because of the possible complicating factor, catamenia. The blood was drawn under sterile conditions after a fast of fifteen hours. It was divided into two parts, and an equal quantity placed in each of two sterile oxalate tubes. It was taken immediately to the laboratory where the sample was centrifuged at 30,000 r.p.m. for fifteen minutes, the plasma removed, and the first cholesterol estimation started within a half hour after the withdrawal of the blood. One tube of blood was kept at room temperature (21 to 24° C.) and the other at icebox temperature (8° C.). Subsequent cholesterol estimations were made on samples of blood drawn from these two specimens at one-, two-, four-, six-, eight-, and twenty-four-hour intervals. The method of determination of the cholesterol was adapted from Bloor,¹² where 1 c.c. of plasma was added slowly with shaking to approximately 40 c.c. of an alcohol-ether mixture

(3 l) in a 50 c.c. volumetric flask. It was then brought to boil on a water-bath, allowed to cool to room temperature, diluted to the 50 c.c. mark, and filtered into an Erlenmeyer flask through fat-free filter paper, the funnel being constantly

TABLE I
ICEBOX GROUP

Showing detailed variations in cholesterol values as measured in mg per 100 c.c. plasma in each individual for each time period, and the gain or loss for eight and twenty four hour periods.

CASE	TIME IN HOURS							GAIN OR LOSS	
	1/2	1	2	4	6	8	24	8 HR	24 HR
1	153	150.0	155.0	151.75	151.75	155.0	156.0	18	-17
2	170	167.5	168.7	166.0	161.25	161.25	129.0	-9	+9
3	200	197.5	197.5	200.0	195.0	221.7	217.5	+23	+17
4	153	156.2	151.2	150.0	156.7	156.2	202.5	+1	+47
5	208	208.7	192.5	192.5	195.0	198.7	210.0	-10	+2
6	210	217.0	217.5	221.4	206.2	211.2	206.2	+1	-4
7	213	213.7	215.0	213.7	217.5	211.2	217.5	2	+4
8	130	131.2	130.0	131.2	122.0	135.0	135.0	+3	+5
9	206	206.2	200.0	200.0	200.0	167.5	163.75	-39	-37
10	213	213.75	217.5	217.5	217.5	196.2	192.5	17	-21
11	185	185.0	186.25	183.0	183.0	168.75	168.75	-17	-17
12	132	128.75	128.75	128.75	140.0	141.25	140.0	+9	+8
13	153	151.25	153.75	153.75	147.5	142.5	142.5	-11	-11
14	158	158.75	158.75	156.7	192.5	192.5	157.2	+34	-1
15	252	242.5	231.25	231.25	243.0	245.0	210.0	-7	-42
16	161	160.25	163.75	161.25	158.75	165.0	192.5	+4	+31
17	148	148.75	150.0	151.25	150.0	183.0	183.0	+35	+35
18	101	161.25	153.75	162.5	161.25	158.75	170.0	-3	+9
19	206	206.2	206.2	210.0	217.5	218.75	215.0	+12	+9
20	176	176.25	176.25	176.25	178.25	190.0	190.0	+14	+14
21	161	161.25	166.25	158.37	166.25	166.25	190.0	+5	+29

ROOM TEMPERATURE GROUP

CASE	TIME IN HOURS							GAIN OR LOSS	
	1/2	1	2	4	6	8	24	8 HR	24 HR
1	153.75	147.50	151.25	151.25	152.5	136.2	133.75	-17	-20
2	170.0	166.25	166.0	157.75	163.75	163.75	121.37	-7	-49
3	200.0	201.2	206.2	203.0	205.0	213.5	242.6	+14	+42
4	155.0	153.7	151.2	151.7	153.0	157.5	200.0	+2	+45
5	208.7	210.0	198.7	200.0	205.0	193.7	213.0	-15	+5
6	210.0	211.2	225.0	217.5	221.2	217.5	205.0	+7	-5
7	213.7	213.7	213.7	213.7	211.2	210.0	223.7	-3	+10
8	130.0	131.2	135.0	131.2	130.0	135.0	131.2	+5	+1
9	206.2	206.2	201.25	202.75	200.0	196.25	172.5	-10	-34
10	213.0	213.75	217.5	217.5	217.5	200.0	185.0	13	-28
11	185.0	185.0	185.0	183.0	187.5	168.75	168.75	-17	-17
12	132.5	128.75	128.75	128.75	140.0	142.5	140.0	+10	+8
13	153.75	153.75	153.75	153.75	147.5	145.0	147.5	-8	-6
14	158.75	158.75	156.2	158.75	195.0	191.7	142.7	+35	-16
15	252.5	245.0	228.0	228.0	252.5	228.0	211.25	-24	-41
16	161.25	161.25	161.25	161.25	160.25	166.25	192.5	+5	+31
17	148.75	148.75	153.0	151.25	151.25	182.5	182.5	+34	+34
18	161.25	161.25	158.75	158.0	161.25	162.5	172.5	+1	+11
19	206.2	206.2	206.2	206.2	206.2	217.5	218.75	+11	+12
20	176.25	176.25	176.25	178.75	176.25	196.25	192.5	+20	+16
21	161.25	161.25	161.25	163.75	161.25	166.25	190.0	+5	+29

covered to prevent evaporation. Twenty cubic centimeters of this filtrate were evaporated just to dryness at a temperature of 60 to 70° C. The residue was extracted 3 times with hot chloroform, using first 5 c.c., then 3 c.c., then 2 c.c.

of chloroform, and transferring each portion to a 10 c.c. graduated, stoppered cylinder. The extractions were made up to 5 c.c. with chloroform. In a similar cylinder 5 c.c. of the chloroform-cholesterol standard containing 0.1 mg. of cholesterol was pipetted. To both standard and unknown there was added 2.0 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulfuric acid. Each cylinder was stoppered and mixed and permitted to stand in the light of the colorimeter for twenty-five minutes. The resulting yellow green colors were compared in the colorimeter, and the cholesterol per 100 c.c. of plasma calculated from the formula:

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 125 = \text{mg. cholesterol per 100 c.c. of plasma.}$$

TABLE II
ICEBOX GROUP

Showing range of group values and average of group values for each time period the cholesterol was measured, and average gain or loss and range of gains or losses for entire group for eight- and twenty-four-hour time periods.

TIME (HOURS)	RANGE	AVERAGE	AVERAGE GAIN OR LOSS	RANGE GAIN OR LOSS
1/2	120-252	176		
1	148-242	177		
2	128-231	176		
4	128-231	176		
6	122-245	179		
8	135-245	179	+0.47	+35 to -39
24	129-217	179	+0.90	+17 to -42

ROOM TEMPERATURE GROUP

TIME (HOURS)	RANGE	AVERAGE	AVERAGE GAIN OR LOSS	RANGE GAIN OR LOSS
1/2	120-252	176		
1	128-245	177		
2	128-228	177		
4	128-228	178		
6	130-252	180		
8	135-228	180	+1.6	+35 to -17
24	121-242	179	+1.3	+15 to -49

Each estimation was done in duplicate and the average value recorded in each instance. The error of the method was considered to be ± 5 mg.

RESULTS

The total number of men in the study was 21. The age range was 23 to 35 years.

Table I shows the detailed cholesterol values in each individual for each time period as well as the eight- and twenty-four-hour gain or loss.

Table II shows the range of gain or loss and average value of cholesterol for the entire group for each time period as well as the average gain or loss and range of gain or loss for the entire group for each time period.

From these tables it can be seen that:

1. Excepting for minor deviations, the change in cholesterol was the same in the icebox group as in the room temperature group.
2. There was generally a change in the cholesterol in a positive or negative manner, with increasing passage of time.

- 3 The greater changes usually occurred after eight hours had lapsed
- 4 The direction and degree of the change could not be predicted
- 5 The average value of change for the groups paralleled closely but were poor indices of the actual change in quantity of cholesterol in the individual case
- 6 The cases in which cholesterol increases occurred were remarkably well balanced by the cases in which decreases occurred
- 7 In some instances the change in the quantity of cholesterol occurred early, while in others it occurred not at all or only very late
- 8 The original quantity of cholesterol in the plasma does not affect the rate nor degree of change in cholesterol as time lapses
- 9 No relationship could be established between the rate of change in the quantity of cholesterol and age, body type, erythrocyte count, leucocyte count, differential count, fluid intake or fluid output

DISCUSSION

The results of this study would indicate that if there is a change in the proportions of the combined and free forms of cholesterol over a twenty four hour period at room or icebox temperatures this change does not always result in a total cholesterol which is identical with the original. In some cases this value remained the same, but there are others in which it was increased or decreased. These increases or decreases moreover, cannot be explained on the basis of an oxidation-reduction action taking place because the values in the icebox group closely paralleled those in the room temperature group. There remain therefore, only three possible explanations for these increases and decreases, i.e., that (a) the anticoagulant acted in some physical or chemical manner to induce them, (b) cholesterol was in some cases increasingly adsorbed or enmeshed in protein molecules, while in others it was progressively released from these molecules and (c) that in some instances the cholesterol was destroyed while in others it was apparently increased by the liberation of bodies in the plasma capable of giving a cholesterol reaction.

Although it is possible that some of the cholesterol in our experiments was destroyed, it is rather improbable that bodies were liberated in the plasma capable of giving a cholesterol reaction. For this reason we feel that the possibility of the latter part of (c) playing a role can be relatively safely ignored. Boyd and Murray,¹³ however, have pointed out that although the anticoagulants heparin, hirudin, and defibrination do not materially alter the cholesterol content of blood plasma, the concentration of cholesterol is invariably lower immediately after the addition of potassium oxalate and depends upon the concentration of the anticoagulant salt and the time over which it acts. In experiments designed to show the effect of potassium oxalate over a period of time from fifteen minutes to three days they found that the oxalate produced an immediate decrease in cholesterol which decrease remained low for six to eight hours on the average, and then returned to the initial level again within twenty four hours. So, it is possible that some of our cholesterol decreases should be explained upon

such a basis. If this is true, then it would be highly advisable to discard the oxalate as an anticoagulant and substitute for it some other anticoagulant, such as heparin or hirudin. This, however, does not explain our increased cholesterol values, and probably not all of the decreased ones. The work of Bruger¹⁴ should also be of some assistance in this respect. He has shown that in ultrafiltration of pleuritic and ascitic fluids through a cellophane membrane the relative concentration of total protein and of free and ester cholesterol in the ultrafilter residue is not significant, excepting that more free than ester cholesterol is left in the residue. Using a Berkefeld (kieselguhr) filter, he found that the filter adsorbs cholesterol selectively, removing approximately 72 per cent of the cholesterol and only 18 per cent of the total protein. There was a definite increase in the ester fraction of the cholesterol in the Berkefeld filtrates. Then Bruger mixed kieselguhr powder with body fluids and found that the powder showed practically the identical results of the Berkefeld filter in that the kieselguhr powder possesses a selective adsorption for free and ester cholesterol, that the adsorption varied directly with the duration of contact. From his work, which supports that of Went and Goreczky¹⁵ and Bendien and Snapper,¹⁶ he pointed out that (a) there may be a stronger linkage between protein and ester cholesterol than between protein and free cholesterol, (b) cholesterol may be adsorbed to protein and thus rendered impermeable, and (c) cholesterol may occur in such fluids as large molecular aggregates which could not readily pass the membrane pores. It is very possible that, as he suggests, the cholesterol is not bound to the protein, but that the large cholesterol aggregates are enmeshed in comparatively large protein particles. This would support the view of Gardner and Gainsborough¹⁷ and Young¹⁸ who feel that cholesterol is bound in a loose way to protein and that no chemical union exists between them. It is conceivable that in our cases where cholesterol increases were found with increased passage of time there was a gradual dissolution or freeing of the enmeshed cholesterol micellae from the protein molecule, with a resulting increase in the total measurable cholesterol.

The problem of the rate of change of cholesterol in a specimen of blood plasma over a period of hours is, therefore, unquestionably a complicated one, and the result depends entirely upon the direction of equilibrium established by the involved factors; i.e., (a) the attempt of a cholesterol esterase to change free cholesterol to a combined form, (b) simple dissolution of cholesterol molecules by hydrolysis, (c) the types of anticoagulant used, and (d) the rate at which the cholesterol aggregates are able to free themselves from the meshes of protein molecules. The resulting progression of events determines whether the cholesterol curve over a period of hours ascends, remains flat, or descends.

SUMMARY

A study of the rate of change of total cholesterol in blood plasma of normal men over a period of twenty-four hours is presented. Changes do occur in positive and negative directions, making group average values over this period of no value. No relationship between this change and other available data was established.

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PENTOTHAL STUDIES WITH SPECIAL REFERENCE TO THE ELECTROCARDIOGRAM*

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AMONG the ultra short acting barbiturates introduced into the field of surgical anesthesia, the group of the so called thiobarbiturates has aroused considerable interest. Certain properties of one of these, ethyl methyl butyl thiobarbituric acid, commonly called pentothal and described first by Volwiler and Tabern,¹ will be discussed in this paper. Weiner, Pratt and Tatum² have very recently made a comparative experimental study of this drug and similar ones, with special reference to their toxicity.

Gruhzit, Dow, Rowe, and Dodd³ reported also recently their investigation of several thiobarbiturates regarding their action upon respiration and blood pressure. One of their statements that "induction of sulphur into Pentobarbital (so forming Pentothal) did not change its anesthetic or toxic properties" can hardly be agreed upon in view of the clinical experience and the results of other

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investigators. Werner⁴ demonstrated in rabbits an interesting difference between evipal and pentothal. The blood pressure fell in the majority of cases following the intravenous administration of evipal, while pentothal produced a rise in pressure in a considerable number of the animals used.

Gruber⁵ drew first attention to his observation that thiobarbiturates produced changes in the electrocardiogram of dogs. In a second paper⁶ the same author published his extensive studies of the action of thiobarbiturates upon respiration and heart. Volpitto and Marangoni⁷ have published work of a similar nature in human beings. Since the pertinent literature on pentothal and its analogues has already been reviewed, we refrain from quoting them again.

Gruber found that the first intravenous injection of pentothal produced disturbances in the electrocardiogram in nearly all cases in dogs, and that the second injection was invariably followed by such disturbances. These changes which he describes consist of alternate ventricular rhythm, premature contractions, and shortened P-R intervals; similar observations were made in cats, monkeys, and rabbits. Morphine enhances the appearance of these disturbances, while atropine does not have any influence. Epinephrine removes them either permanently or temporarily. Before this last paper of Gruber's was published, we were performing similar investigations, the results of which are described in this publication.

TECHNIQUE

Dogs, cats, rabbits, and monkeys (*Rhesus macacus*) were used. Electrocardiographic records were taken of all animals with Lead II, using a Cambridge string galvanometer, one millivolt giving 1 cm. excursion of the string.

Silver plate electrodes were applied to the shaved extremities of the animals by means of gauze soaked with saturated sodium chloride solution. In one group of animals no anesthesia was used previous to the first pentothal injection. A control tracing was taken of the unanesthetized animal. In a number of cases the pentothal anesthesia was utilized for preparation of the animal for blood pressure and respiration records. The carotid artery was cannulated for the recording of the blood pressure. Respiration was recorded by means of a tracheal cannula with side tube connected with a Marey-tambour. In another group of animals this preparation was done under light ether anesthesia, and the first pentothal injection was made subsequently after the ether effect had subsided. Finally, several animals were subjected to anesthesia only without any surgical preparation, and their respiration was recorded by means of a pneumograph connected with a Marey-tambour.

Pentothal was used in the form of its sodium salt in a 1 to 5 per cent freshly prepared solution in distilled water. The rate of injection varied intentionally in the different experiments, allowing thirty seconds to three minutes for the administration of a single dose. Evipal was used in some experiments in the same concentrations.

RESULTS

1. *Dogs*.—Six animals, weighing between 7 and 18 kg. were used. Two of these were given light ether anesthesia previous to pentothal. One animal re-

ceived ether for a short time during the experiment. The other three were injected with the barbitamates only. One of these dogs, which was extremely fat, died shortly after a rapid (thirty seconds) injection of 12 mg/kg pentothal. All the other animals received repeated injections. Table I gives a summary of these experiments.

TABLE I
EXPERIMENT ON DOGS

DOG NO & WEIGHT	PRIMARY ANESTHESIA	SECONDARY ANESTHESIA DRUG & DOSE	MINUTES INTERVAL BETWEEN INJECTIONS	REMARKS
1 18 kg	Ether	Pentothal 10 mg/kg Evipal 10 mg/kg Pentothal 10 mg/kg Evipal 10 mg/kg Pentothal 10 mg/kg	18 42 58 50	
2 60 kg	Ether	Pentothal 12 mg/kg		Very rapid injection, died
3 90 kg	Ether	Pentothal 8 mg/kg Pentothal 10 mg/kg Evipal 10 mg/kg Pentothal 10 mg/kg	29 20 14	
4 126 kg	None	Pentothal 10 mg/kg Pentothal 20 mg/kg Evipal 15 mg/kg Pentothal 20 mg/kg	20 25 60	
5 119 kg	None	Evipal 25 mg/kg Pentothal 20 mg/kg	15	10 minutes later 0.5 mg/kg atropine sulfate
		Pentothal 25 mg/kg	45	Followed by 10 minutes ether anesthesia
		Pentothal 20 mg/kg Pentothal until respiratory paralysis	65	
6 120 kg	None	Pentothal 15 mg/kg Pentothal 15 mg/kg Pentothal 15 mg/kg Pentothal 15 mg/kg	21 10 17	25 mg ephedrine sulfate 3 minutes after pentothal
		Pentothal 15 mg/kg Pentothal until respira- tory paralysis	19 8	

Similar to Weiner's findings in rabbits, we found in dogs that pentothal seems to have a more pronounced depressive action upon the respiration, while evipal produces a drop in blood pressure which is not often seen with pentothal. Fig. 1 illustrates this effect of the two drugs upon Dog No. 1. Subsequent injections gave corresponding results. No disturbance in the cardiac rhythm was recorded in the electrocardiogram. The pulse rate was influenced very little and showed occasionally a drop from 240 beats to 230 beats per minute after pentothal injection. There was no appreciable change in the P-R interval which was $\frac{7}{5}$ of a second.

Dog No. 2, which ceased breathing immediately after the first dose of pentothal, showed no significant changes in the electrocardiogram until it died by respiratory paralysis.

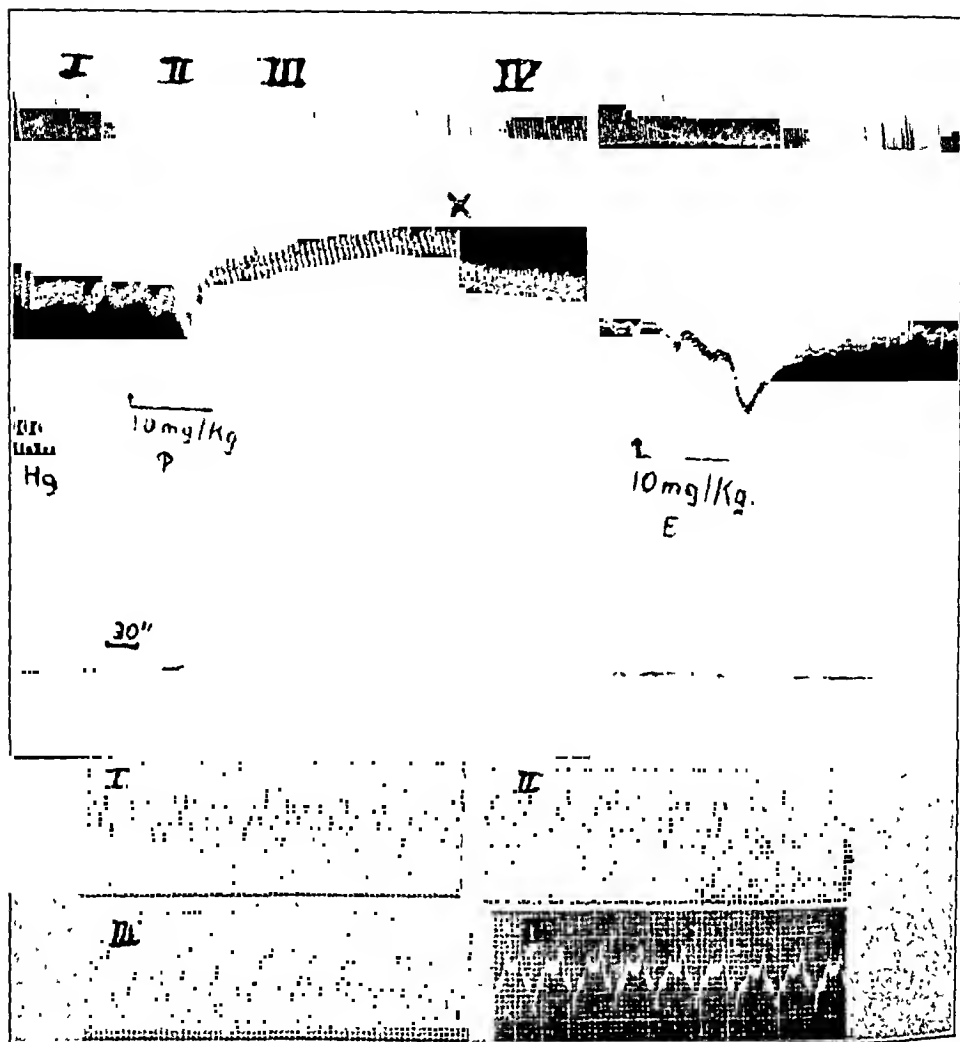


Fig. 1.—Dog No. 1. Upper tracing respiration, lower tracing carotid blood pressure. The pentothal injection and the evipal injection an earlier phase of the experiment. The rate of numbers in the tracings correspond to those of an identical one in for both drugs. The kymographion was stopped for five minutes at x.

I—Forty minutes after last pentothal injection.

II—During injection of 10 mg./kg. pentothal.

III—Immediately after injection.

IV—Ten minutes after injection.

No significant changes in the electrocardiogram. The tracings after evipal (E) were also normal.

Dog No. 3 showed no marked change in pulse rate and electrocardiogram after the administration of either pentothal or evipal. Repeated tracings were taken before, during the injection, and afterwards (see Fig. 2A).

Dogs No. 4 and 5 showed normal electrocardiograms following the injection of evipal, except for a depression of the T-wave. However, the administration

of pentothal was followed by changes in the electrocardiogram after every injection in these two dogs. The time of onset of the disturbances varied from one to six minutes. It was noted that in all cases the changes subsided with the end of the anesthesia. Some typical tracings are given in Fig 2B, C and their inter-

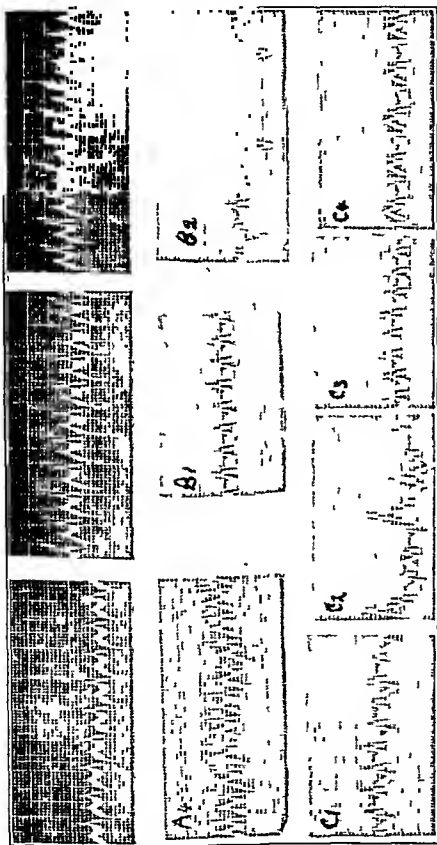


Fig. 2.—A—Dog No. 3. A1, control tracing thirty nine minutes after a previous injection of pentothal. A2, immediately after 10 mg/kg pentothal. A3, five minutes later. A4, sixteen minutes later, dog, awakening. No irregularities in the electrocardiogram. Slight shortening of the P-R interval.
B—Dog No. 4. Two injections of pentothal and one of atropine were given previously. B1, sixty minutes after last injection, animal awake. B2, three minutes after 20 mg/kg pentothal, normal complexes with markedly T-wave and also abnormal beats which later show a shortened P-R interval and probably disturbances in the intra ventricular conduction.
C—Dog No. 5. One injection of atropine and one of pentothal were given previously. C1, forty-five minutes after last pentothal injection and ten minutes after 6 mg atropine sulfate. Intravenously. Dog, awake, rapid pulse rate. P-wave depressed. C2, three minutes after 21 mg/kg pentothal. Normal and abnormal beats, the latter showing different types and a very short P-R interval. The T might be of nodal origin. C3, Dog, awakening, electrocardiogram normal. C4, six minutes after the last injection of 20 mg/kg pentothal. Atrial fibrillation rhythm between normal and abnormal complexes with very short P-R interval and inversion of the ventricular complex. These may also be of nodal origin. The electrocardiogram was within normal with the fading of the anesthesia.

pretation described in the legend. In concordance with Gruber's experience, atropine did not influence the appearance or duration of the disturbances.

The experiment conducted on Dog No. 6 did not show any pathologic changes in the electrocardiogram. The injection of 25 mg of ephedrine sulfate during pentothal anesthesia produced a marked sustained rise of the blood

pressure. However, it did not provoke the appearance of cardiac irregularities. The injection of pentothal was finally continued until complete and permanent stop of respiration occurred. No cardiac disturbances appeared even after this toxic dose.

2. *Cats and Rabbits.*—Six cats (2 to 3.8 kg.) and two rabbits (2 to 3.6 kg.) were used; the technique was identical as that used in dogs (Table II).

TABLE II
EXPERIMENT ON CATS AND RABBITS

ANIMAL NO. & WEIGHT	PRIMARY ANESTHESIA	SECONDARY ANESTHESIA DRUG & DOSE		INTERVAL BETWEEN INJECTIONS	REMARKS
Cat 1 3.0 kg.	Ether	Pentothal	20 mg./kg.		Rapid injection (30 seconds); death by respiratory paralysis
Cat 2 2.0 kg.	Ether	Pentothal Pentothal	10 mg./kg. 15 mg./kg.	26 min.	Rapid injection (30 seconds); respiration markedly depressed
Cat 3 2.3 kg.	None	Pentothal Pentothal	16 mg./kg. 18 mg./kg.	38 min.	Both injections were made rapidly. The respiration ceased for a short time
Cat 4 2.6 kg.	None	Pentothal Pentothal Pentothal Pentothal until respiratory paralysis	15 mg./kg. 15 mg./kg. 12 mg./kg. 12 mg./kg.	15 min. 68 min. 16 min.	7 mg. ephedrine sulfate 11 min. after first pentothal injection; 5 mg. ephedrine combined with second. Animal received 0.15 mg./kg. K-strophanthin 20 min. previous to this pentothal injection
Cat 5 3.8 kg.	None	Pentothal	25 mg./kg.		Animal previously submitted to repeated chloroform anesthesia (see text) Additional chloroform anesthesia Death from respiratory paralysis
		Pentothal	15 mg./kg.	30 min.	
		Pentothal	13 mg./kg.	12 min.	
Cat 6 2.4 kg.	None	Pentothal	20 mg./kg.	5 min.	Treated with thyroxine (see text) 3 min. artificial respiration after first pentothal injection
		Pentothal	18 mg./kg.		
		Pentothal	12 mg./kg.	41 min.	
Rabbit 1 2.0 kg.	None	Pentothal	10 mg./kg.	8 min.	
		Pentothal	10 mg./kg.	9 min.	
		Pentothal	30 mg./kg.	15 min. 10 min.	
Rabbit 2 3.6 kg.	None	Evipal	30 mg./kg.		2 min. were used for each injection; pentothal was more depressant upon respiration than evipal
		Pentothal	20 mg./kg.	20 min.	
		Evipal	30 mg./kg.	32 min.	

Cat No. 1.—Several electrocardiogram tracings were taken before and after the injection of pentothal. The cat died following a rapid injection of 20 mg./kg. due to respiratory paralysis. The electrocardiogram did not show disturbances in rhythm or conduction.

Cat No. 2 showed a normal electrocardiogram following the injection of pentothal (Fig. 3A).

Cat No. 3.—No disturbances in the electrocardiogram occurred.

Cat No 4 showed cardiac disturbances (Fig 3B). These followed every injection of pentothal in this animal. The disturbances developed about three minutes after the injection. There first appeared single premature beats of

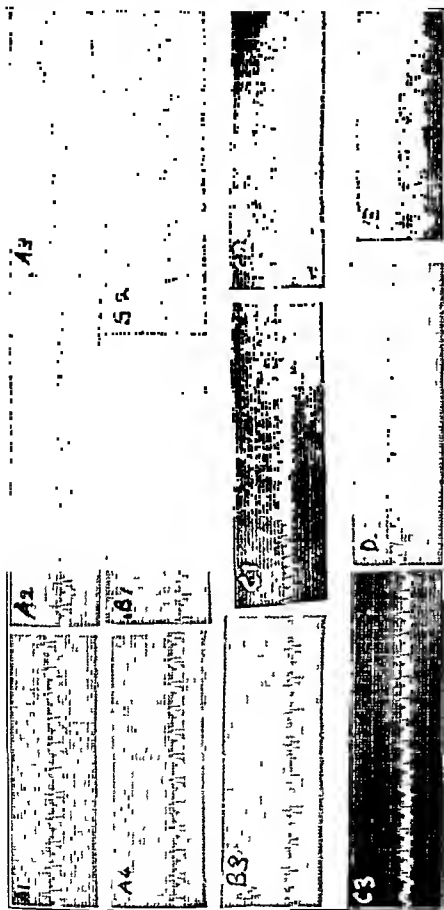


Fig. 3—1—Cat No. 2. After sixteen minutes after a previous pentothal injection. T-wave depressed. A2, immediately after 15 mg/kg pentothal. A3, 4, 5 ten minutes later. Pulse rate and electrocardiogram not significantly influenced. B—Cat No. 4. B1, normal control pulse rate 210. B2, five minutes after 15 mg/kg pentothal. Alternating normal and ventricular premature beats. B3, one minute after 7 mg/kg ephedrine sulfate injected during the state of cardiac disturbances. Normal rhythm restored. Pulse rate 240.

C—Cat No. 5. C1, fifty minutes after first pentothal injection. Cat awake (Time marker not working). C2, animal under chloroform anesthesia and one minute after 13 mg/kg pentothal. Note normal beats with shortened P-R interval and abnormal ventricular beats. A subsequent injection of 20 mg/kg pentothal caused respiratory paralysis which was fatal. C3 shows the return of a normal electrocardiogram during the first state of respiratory paralysis. The rhythm remained normal until death. T-wave negative in all tracings.

D—Rabbit No. 2. Electrocardiogram following 20 mg/kg pentothal shows one series of alternation between normal and premature ventricular beats. Another such attack followed and a normal rhythm was reestablished.

E—Monkey No. 5. This animal previously had 5 mg/kg morphine sulfate and 0.5 mg/kg atropine sulfate. Electrocardiogram following 10 mg/kg pentothal. The atypical beats appearing in groups of two in this tracing are not premature beats but are probably due to interference with the intraventricular conduction.

ventricular origin. These were followed by an alternating normal and premature rhythm. All changes were completely removed by the intravenous injection of 7 mg ephedrine sulfate. However, the disturbances as first seen reoccurred after a second injection of 15 mg/kg pentothal combined with 5 mg ephedrine

sulfate. The electrocardiogram returned to normal eighteen minutes after this injection. The same cat received repeated injections of K-strophanthin, amounting to 0.15 mg./kg., which produced marked changes in the electrocardiogram. These were irregularly premature ventricular beats. Twelve milligrams per kilogram of pentothal, injected at this time, increased the number of extrasystoles which was later followed by alternating normal and premature beats. The P-R interval was shortened in the normal beats. However, sixteen minutes after the injection of the pentothal, the arrhythmia subsided completely together with the fading of the anesthesia. A toxic dose of pentothal killed the animal by respiratory paralysis. Here also, as observed before, the disturbances which appeared after this last injection disappeared again long before the death of the animal.

Cat No. 5 was subjected every day for four days previous to the experiment to chloroform anesthesia of fifteen minutes duration. The first injection of pentothal in this animal was followed by a few ectopic beats, which soon disappeared completely in favor of a normal rhythm. These disturbances were more pronounced after the second injection. After normal rhythm was restored and the narcosis subsided, chloroform anesthesia was introduced and 30 mg./kg. pentothal were injected. Fig. 3C shows the appearance of ectopic beats after pentothal. The T-depression present before the injection is questionable in its significance. The electrocardiogram became normal about eleven minutes after the injection. Death followed a second injection of 20 mg./kg. pentothal. This dose first caused an arrhythmia, but the rhythm became normal long after the respiration had ceased.

Cat No. 6 was treated for eight subsequent days previous to the experiment with thyroxine injection totaling to 8 mg. The weight of the cat dropped from 2.8 kg. before this treatment to 2.4 kg. The heart rhythm and the electrocardiogram remained completely undisturbed after all pentothal injections.

Generally the pulse rate dropped 20 to 40 beats per minute in cats after pentothal injection. The respiratory depression was more marked than in dogs.

Rabbit No. 1 did not show changes in the electrocardiogram after the first two pentothal injections. The third injection was followed by a marked respiratory depression which necessitated artificial respiration. A few beats of ectopic origin occurred following this dose.

Rabbit No. 2.—This animal gave a normal electrocardiogram following the first 30 mg./kg. of evipal. This amount was injected in two minutes and caused a temporary stop of respiration. Twenty milligrams per kilogram pentothal given twenty minutes later produced a longer stop of respiration, but the animal recovered after artificial respiration was administered. An alternation between normal and ventricular premature beats appeared about three minutes after the injection (Fig. 3D). This rhythm occurred in attacks of 10 to 20 beats three times and was finally replaced by a permanent and normal rhythm. The last evipal injection of 30 mg./kg. produced no change in rhythm but a lengthened P-R interval, from $\frac{2}{25}$ to $\frac{1}{25}$ of a second. The pulse rate was either not influenced or dropped after both barbiturates.

3 Monkeys—Five animals (22 to 32 kg) were used. Two of these were prepared for recording of blood pressure and respiration after an anesthesia was induced through the first or second injection of pentothal.

Monkey No. 1—There was no change in rhythm or P R interval in any of the tracings. The T wave was depressed and the pulse rate dropped slightly.

Monkey No. 2—Twenty milligrams per kilogram of pentothal injected within thirty seconds produced cessation of respiration. The animal was revived by artificial respiration. No significant change in the electrocardiogram tracings occurred. The pulse rate increased for a short time. The second injection of 12 mg/kg did not bring about any disturbances in rhythm.

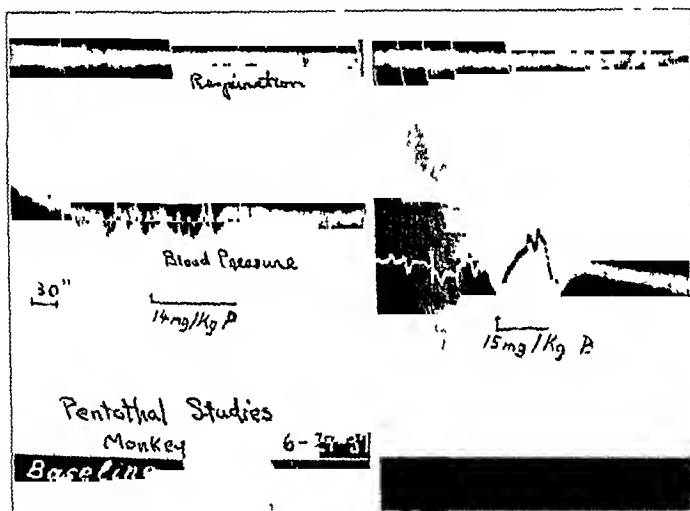


Fig. 4—Monkey No. 4. This animal has already had three pentothal injections which totaled 30 mg/kg.

I—Forty-two minutes after last injection 14 mg/kg pentothal (P) in ninety seconds.

II—Thirty minutes after I injection of 15 mg/kg pentothal in sixty seconds.

Note the difference in the effect upon the blood pressure according to the speed of the injection. The electrocardiogram remained normal in all tracings.

Monkey No. 3 (recording of blood pressure and respiration) showed a moderate drop of blood pressure after the relatively fast injection (one minute) of 12 mg/kg pentothal. The electrocardiogram was not significantly influenced by any of the pentothal injections done in this animal.

Monkey No. 4—The second injection (15 mg/kg) was used for preparation of carotid artery and trachea. Repeated injections of pentothal were given, but no pathologic disturbances in the cardiac rhythm occurred. As can be seen in Fig. 4, the slow injection of 14 mg/kg did not influence the blood pressure. The respiration remained practically unchanged. A second injection, given

somewhat faster, produced a slight drop, secondary rise, and finally a leveling off of the blood pressure. The rate of respiration was slightly decreased following the second injection.

Monkey No. 5.—This animal received morphine atropine thirty minutes previous to the first pentothal injection. The effect of the barbiturate was very much prolonged as compared with other animals. The electrocardiogram taken after pentothal injection showed the appearance of grouped beats with disturbances in conductivity as demonstrated in Fig. 3. The pulse rate dropped from 180 to 150 beats per minute. The second injection, however, of 8 mg./kg. was not followed by any such disturbances.

TABLE III
MONKEYS
NO PRIMARY ANESTHESIA

ANIMAL NO. AND WEIGHT	DRUG AND DOSE	INTERVAL BETWEEN INJECTIONS	REMARKS
1 3.2 kg.	Pentothal 15 mg./kg. Pentothal 15 mg./kg.	26 min.	
2 2.3 kg.	Pentothal 20 mg./kg. Pentothal 12 mg./kg.	50 min.	Rapid injection (30 seconds) caused respiratory depression
3 2.2 kg.	Pentothal 12 mg./kg. Pentothal 20 mg./kg. Pentothal 12 mg./kg.	8 min. 36 min.	
4 3.0 kg.	Pentothal 12 mg./kg. Pentothal 15 mg./kg. Pentothal 12 mg./kg. Pentothal 14 mg./kg. Pentothal 15 mg./kg.	9 min. 20 min. 40 min. 30 min.	Rapid injection (see Fig. 4)
	Pentothal.	Injection until respiratory paralysis, subsequent death.	
5 2.6 kg.	Pentothal 10 mg./kg.		5 mg./kg. morphine sulfate and 0.5 mg./kg. atropine sulfate 30 min- utes before pentothal injection
	Pentothal 8 mg./kg.	26 min.	

DISCUSSION

We shall confine our discussion to the electrocardiographic findings in our experiments. It is interesting to note that in a number of animals no disturbances whatsoever occurred, while in others every injection provoked them. Initial ether anesthesia seems somewhat to decrease the appearance of disturbances in dogs and cats. However, we do not wish to say if this is an accidental observation or if a definite connection exists, especially since it is hard to believe that the effect of a slight ether anesthesia, which has been discontinued very early in the experiment, would influence the reaction of the animal for several hours. Ether, if given after the appearance of disturbances, was not able to suppress these changes. The nature of the changes in the electrocardiogram consisted chiefly of disturbances in the intraventricular conduction and the appearance of beats of ectopic origin. It is remarkable that often a normal rhythm was present as long as six minutes after the injection before the first signs of disturb-

ances occurred. In one instance we observed beats with a sharply inverted T-wave, followed by conduction disturbances and finally extrasystoles.

Like Gruber, we occasionally noted in dogs alternation of normal and ectopic beats. The shortening of the P-R interval in dogs occurred more rarely in our experiments than in those of Gruber. We were not able to find rhythm disturbances as regularly as reported by Gruber. On an average our doses were smaller than his. Occasionally small doses (10 mg/kg) were followed by disturbances, whereas larger ones would quite frequently not produce any such changes. Gruber found premature beats in dogs following 10 mg/kg pentothal. It is important to note that large doses, which caused the deaths of the animals, did not bring about any disturbances in the electrocardiogram or, if such occurred, they disappeared a considerable time before death. Death was in all instances due to respiratory paralysis; we observed neither a primary heart death nor the development of ventricular fibrillation. The occurrence of ectopic beats following the introduction of anesthesia in experimental animals has been observed by Came and Reynolds⁸ following propylene administration. Kahn and Riggs,⁹ however, found no such disturbances in man with the same anesthetic. SeEVERS, Meek, ROVENSTINE and STILES¹⁰ anesthetized dogs with cyclopropane and found nodal beats, heart block, premature contractions, fibrillation, and death. This can happen in the presence of a sufficiently high oxygen tension. Volpitto and Marangoni have published their electrocardiogram findings in pentothal anesthesia in human beings and found neither disturbances in rhythm nor any influence upon such already present. We tried as described above, to damage artificially the heart in cats by repeated chloroform anesthesia, stiophanthin, or with thyroxine. We found that even in the case where stiophanthin was used and began to produce marked changes in the electrocardiogram pentothal caused the typical disturbances of its own and the electrocardiogram became normal again a few minutes later. In all cases the normal rhythm returned with the end of the anesthesia. Similar to Gruber's findings with epinephrine, we noticed that ephedrine is sometimes able to remove the rhythmic disturbances. Large doses, which greatly favor ectopic formation of stimuli, did not sensitize the heart so that pentothal caused irregularities. It is most interesting to note that in our experiments monkeys, being primates, did not show any cardiac disturbances after pentothal was given in moderate doses, except following a morphine premedication. In man, according to the newest publication of ROVENSTINE's school, no disturbances occurred after pentothal even when used in conjunction with morphine.

SUMMARY

1 The effect of the intravenous injection of pentothal sodium in dogs, cats, rabbits, and monkeys was studied with special attention to the electrocardiogram. Evipal was used in some instances for comparison.

2 A large percentage of the animals used in our experiments showed no significant changes in the electrocardiogram following the injection of pentothal.

3 Another group of animals following pentothal injections reacted with disturbances in the electrocardiogram, such as ectopic beats, alternating normal and premature rhythm and disturbances in the intraventricular conduction.

The P-R interval was shortened in some of the animals. Evipal elicited a marked increase in the P-R interval in one rabbit. Depression of the T-wave may follow both barbiturates.

4. Treatment with chloroform, strophanthin, or thyroxine in cats did not influence the appearance or duration of electrocardiographic disturbances. Atropine did not overcome these disturbances, while ephedrine may remove them occasionally.

5. Electrocardiographic disturbances, if present, disappeared without exception with the end of the anesthesia. Deaths following a large dose or a too rapid injection of pentothal occurred always from respiratory paralysis. Present disturbances in the electrocardiogram due to pentothal subsided without exception before the deaths of the animals.

6. Pentothal in moderate doses did not produce any significant changes in the electrocardiogram of monkeys, except after morphine premedication. The blood pressure of monkeys was not influenced by a slow injection of the drug.

While this paper was in print, Charles J. Betlach (*J. Pharmacol. & Exper. Therap.* 61: 329, 1937) reported that he found no significant changes in the electrocardiogram of dogs following the administration of either amytal or pentothal.

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LABORATORY METHODS

LABORATORY DIAGNOSIS IN CHRONIC GONORRHEA OF THE FEMALE*

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THE routine Gram stain for diagnosis of acute gonorrhea is less helpful in chronic gonorrhea because of scarcity of the organisms in the discharge and their extracellular position. The value of the cultural method is lessened by the fact that due to the peculiar physiology of the gonococcus more time and skill are required than are routinely practicable. Out of these difficulties has arisen the recognition for the need of a test which is simple, definite, and specific. The complement fixation test would seem to fulfill these requirements. The aim of this work was to determine the value of smear culture and complement fixation as routine diagnostic aids in chronic gonorrhea of the female.

LITERATURE

The gonococcus has long been suspected of being microaerophilic, and the sites it chooses to survive in, within the body, seem to bear out this view.

Wherry and Oliver¹ were the first to culture the gonococcus in the presence of reduced oxygen tension by using Nowak's method of culturing in conjunction with *B. subtilis*. Ruediger² obtained good growth by using rubber stoppers in place of cotton plugs and concluded that reduction of oxygen tension, rather than the presence of carbon dioxide, was the important factor. Rockwell and McKhann³ obtained growth in pure hydrogen but not under the anaerobic condition produced by caustic soda and pyrogallie acid. McLeod and his associates,⁴ Leahy and Carpenter,⁵ Nye and Lamb,⁶ and Luther Thompson⁷ find the presence of 8 to 10 per cent carbon dioxide beneficial. Thompson produces the carbon dioxide by placing 10 c.c. of an 8.4 per cent solution of sodium bicarbonate in a beaker along with the inoculated plates in a museum jar and then pipettes 10 c.c. of 33 per cent sulphuric acid through a hole in the lid and seals the hole.

Schultze⁸ first observed the effects of alpha naphthol and dimethyl para-phenylenediamine on bacteria. Gordon and McLeod⁹ poured 1 per cent dimethyl-paraphenylenediamine hydrochloride over their cultures and found that it differentiated between *V. cholera* and *B. coli*. The former turns pink (Winstler's red) and then within a few minutes becomes black, while the latter does not change.

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color. The reaction likewise differentiates between gonococci, staphylococci, and streptococci. Along with the gonococcus other positive oxidase reactors are: *V. cholera*, *N. intracellularis*, *N. catarrhalis*, *N. flava*, *B. subtilis*, *B. anthracis*, *B. pyocyaneus*, *H. influenzae*, and *H. pertussis*. Most of these, however, can be easily differentiated from the gonococcus by the appearance of their colonies.

Dimethyl-paraphenylenediamine hydrochloride turns oxidase positive colonies first pink, then gradually black within fifteen minutes. When the colonies are black, the bacteria have been killed. Price¹⁰ finds a pseudoreaction turning brownish pink to brownish black. Some workers prefer the tetramethyl compound because it does not kill the bacteria as soon. It turns oxidase positive colonies deep purple, rather than black, and is more sensitive, giving positive reactions with *B. influenza* and *H. pertussis*. However, it darkens the media quickly, making observation difficult and is much more expensive. The method now in use is to pour a few cubic centimeters of a 1 per cent dimethyl compound, or 0.5 per cent tetramethyl compound, over the plate and immediately pour it off again. The solution darkens in a few hours at room temperature, but remains useful for several days. Leahy and Carpenter¹² use the dimethyl compound. McLeod and his coworkers, Thompson,¹³ and Spohr and Landy¹⁴ use the tetramethyl compound. Those who use the dyes agree that they are very useful in detecting colonies of gonococci, and it is significant that 14 per cent of the positive cultures found by Leahy and Carpenter were detected only by the use of the oxidase test and would have been missed otherwise.

Since other bacteria present in the cultures may give the oxidase reaction, most workers stain and examine the oxidase positive colonies and identify further by fermentation reactions. Elser and Huntoon¹⁵ established the fact that the gonococcus ferments dextrose, but not maltose, while *N. intracellularis* ferments both and *N. catarrhalis* neither. Cole and Lloyd¹⁶ found some fermentation of galactose as well as glucose, but Torrey and Buckell¹⁷ could not substantiate these findings.

Raven,¹⁸ by using immune serum and by aging her cultures, has produced dissociation of the gonococcus which differs in its Gram stain, colony appearance, and fermentation reaction. Herrold,¹⁹ by culturing in unfavorable environment, was also able to produce Gram-positive forms which are of significance in the clinical course of gonorrhea. Williams and Styron,²⁰ Barringer, Strauss, and Crowley,²¹ examining various causes for cervicitis, found that catarrhalis, streptococci, coccobacilli, *B. influenzae*, diphtheroids, trichomonas, yeast, and actinomyces gave symptoms causing erroneous diagnosis of gonorrhea. Williams and Styron emphasize that the streptococci and coccobacilli existing with or without the gonococcus may complicate any laboratory diagnosis.

McLeod, after examining 2,062 cases, concludes that cultures, especially from the cervix, are superior to smears for diagnosis. He could not explain the residue of cases with positive smears and negative cultures. Leahy and Carpenter obtained 130 positive cultures from 302 specimens, which was 10 per cent more than by the smear method alone. Spohr and Landy with 59 patients found 10 per cent negative smear, but positive culture, and 37 per cent

both positive smear and culture from the urethra, 32 per cent negative smear, but positive culture from the cervix, and 19 per cent both smear and culture positive

Müller and Oppenheim²² were the first to employ the complement fixation test as an aid in differentiating gonococcal arthritis from arthritis due to other causes. Baringer, Struss, and Crowley state that in a previous study of 256 cases of gonorrhea in females the complement fixation test is valuable. Any degree of positiveness indicates gonorrheal infection. It becomes positive three to five weeks after onset of infection, is negative during the acute stage and becomes positive during the subacute stage when smears and cultures are doubtful, and during the chronic state subsides along with bacteriologic and clinical findings. From 200 cases of possible gonorrhea they obtained positive results for 110 by either smear, culture or complement fixation. Ninety five cases were positive by complement fixation and 27 were positive by that test alone. Tulloch²³ states that the complement fixation test in women gives a low positive rate when only the cervix is involved a positive rate of 60 per cent in cases of proctalgia and involvement of Bartholin glands, and usually negative reactions in cases of urethritis. Torrey, Wilson, and Buckell²⁴ found smears of least value, cultures next and the complement fixation test of greatest value in diagnosing chronic gonorrhea.

PROCEDURE

Isolation of the gonococcus was attempted in 100 female cases of chronic gonorrhea. The patients' symptoms fitted in general the clinical criteria, outlined by Smith and Wilson² which are as follows:

Discharge Slight or profuse mucopurulent in character

Bartholin glands Showing fibrous change and purulent discharge

Skene's glands Express pus on massage

Urethra No inflammation apparent

Cervix May or may not be enlarged Canal usually unduly patent

Erosions fairly constant and mucopurulent discharge

Evidence of prior adnexal involvement such as the thickened tubes, loss of mobility of uterus, with evidence or history of previous pelvic or abdominal surgery

Urethral and endocervical smears were taken with applicators and stained by Gram's method. Smears showing Gram negative, intracellular coffee bean shaped diplococci were considered positive, while those showing such organisms only extracellularly were regarded as doubtful or suspicious. Plated culture media were directly streaked from urethra and endocervix with sterile cotton applicators and within two hours were placed in an incubator kept at 35° to 37.5° C. During the first half of the experiment a shallow pan of water was kept in the bottom of the incubator. The pan of water was later discarded and the cultures incubated in lots of six to eight in airtight Novy jars in which 8 to 10 per cent carbon dioxide was produced by mixing sulfure acid with a solution of sodium bicarbonate as in Thompson's method. The routine media used were plain blood agar, chocolate agar, amniotic fluid agar, testicular agar

positive and Gram-negative bacilli, yeasts, and rarely Gram-positive cocci in clumps or pairs become dark, but not as rapidly or definitely as did the gonococci. The dyes were very helpful for a rapid determination of negative plates, but when suspicious small round colonies appeared on a plate, a higher incidence of positive subcultures was obtained by picking off a representative colony before flooding the plate with the dye, and then subculturing if the other similar colonies gave the oxidase reaction. Running the dye over the plate often caused contamination of the gonococcus colonies and, therefore, contaminated subcultures. The percentage positive without the use of the dye was 4 per cent, while the percentage positive with dye and carbon dioxide was 11 per cent.

Preliminary cultures on plain agar showed less growth than on the richer media, and never gave any Gram-negative cocci. Of the 44 cultures on amniotic fluid agar 4 per cent were positive; of 70 on blood agar 4 per cent were positive; on testicular (Difeo) 4 per cent; on chocolate agar 9 per cent; on Douglas agar with sodium acid phosphate plus ascitic fluid 9 per cent; and on Douglas agar plus heated blood 14 per cent were positive. These results are quite indicative, but not conclusive, because the oxidase test and carbon dioxide were never used with plain chocolate, testicular, or amniotic agar.

When the cultures were incubated in the presence of moisture but without carbon dioxide, the percentage positive was about 8.5 per cent; and when incubated in closed jars containing 8 to 10 per cent carbon dioxide, the per cent positive increased to about 15.5 per cent. The beneficial effect of carbon dioxide seems to be clear.

Fermentation media prepared according to Zinsser and Bayne-Jones²⁶ which calls for the addition of sugars to Douglas-ascitic fluid agar, and tested with strains of *B. coli*, *N. catarrhalis*, and a Torrey strain of gonococcus gave their typical reactions. The difficulty in obtaining pure cultures of the gonococcus on fermentation media, and the results of various workers who examined hundreds of cases without finding *N. catarrhalis* or *N. intracellularis* seem to make it unnecessary to attempt routine identification by sugar reactions for confirmatory diagnosis. However, the presence of other Gram-negative diplococci and diplobacilli in smear and culture makes confirmatory diagnosis on fermentation media absolutely necessary in chronic gonorrhea.

DISCUSSION

Since 15 "suspicious" direct smears were unconfirmed by other tests and 19 were confirmed, the difference does not seem great enough to warrant a report of gonococcus positive when only Gram-negative typical coffee-bean extracellular diplococci are present. However, when one elicits a previous history of exposure, the medicolegal aspects place the burden of chronic gonorrhea on the laboratory, because repeated tests are necessary to establish diagnosis.

The value of repeated smears and cultures is shown by the fact that for 17 positive urethral and cervical cultures obtained from 9 patients in Group I, 51 urethral and cervical cultures were taken from these patients, and by the fact that in this same group the percentage of positive cultures was higher than in Group II.

Concerning isolation of the gonococcus from surgical specimens we see that positive smears and cultures are infrequent and rare. The bacteria observed are difficult to describe because of variations in morphology and staining reactions.

The incidence of *B. coli* indicated that its presence in the female genitourinary tract of 13 per cent of the gynecologic patients was due to the proximity of the rectum and faulty hygienic habits. The frequent predominance of other organisms where repeated laboratory tests for gonococci are negative suggests that clinical criteria are apt to be misleading and should always be verified by repeated laboratory tests.

The use of the oxidase test is recommended as an additional aid to isolation rather than as a sole test, and should be used in conjunction with direct observation of the colonies and with the Gram stain.

The marked increase in positive results following use of Douglas agar as a base with heated blood or aseptic fluid and incubated in a moist carbon dioxide atmosphere demonstrates the importance of these factors. Since the chocolate Douglas agar gave a higher percentage of positives with larger colonies of the gonococci than did the aseptic Douglas agar, it is the method suggested for routine use.

CONCLUSIONS

1 Douglas agar with heated blood is the medium of choice for culturing gonococci because of the nutritive value of the Douglas agar and the growth stimulating factors present in heated blood.

2 The presence of carbon dioxide in the incubator atmosphere is of more importance than the presence of moisture in the primary isolation of gonococci by culture.

3 The oxidase test with dimethylparaphenylenediamine hydrochloride serves as a confirmatory test in the isolation of gonococci.

4 Isolation of gonococci from 14 surgical specimens taken from patients with a history of chronic gonorrhea was not successful.

5 For the laboratory diagnosis of chronic gonorrhea in women cultures are superior to direct smears and gonococcus complement fixation tests are superior to either cultures or direct smears.

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CONCENTRATION OF TUBERCLE BACILLI FROM SPUTUM BY CHEMICAL FLOCCULATION METHODS*

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IN VARIOUS experiments which have necessitated the removal of tubercle bacilli from suspensions in water and other fluids, it has been observed that these organisms could not be sedimented with any degree of completeness by prolonged centrifugation in machines of the conventional design and speed. In certain instances this difficulty was overcome by the preparation of alum flocculations in the water with the result that the bacilli were removed quite completely during very brief centrifugation. On other occasions, the bacilli were washed by centrifugation in solutions which approximated the isoelectric point of the organisms (pH 2.8).

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These observations suggested that the concentration of tubercle bacilli from clinical specimens by centrifugation in the usual way might be extremely inefficient and that, possibly, the use of flocculating agents or the isoelectric pH might assist in the collection and demonstration of bacilli from sputa and body fluids which are negative when concentrated by the usual methods.

A preliminary series of tests was made on positive sputa by using 0.5 per cent alum to flocculate the supernatant fluids which are discarded following the customary "centrifugation of digested sputum at high speed for 30 minutes." Microscopic examination of these alum precipitates always revealed that the usual centrifugation had not collected all the bacilli from the supernatant fluids. The alum precipitates often contained more bacilli than the original centrifuge sediments. It was thus clearly demonstrated that direct centrifugation does not collect the tubercle bacilli from sputum completely enough to qualify as a desirable method.

It was the purpose of this study to learn what might be expected in routine clinical work if acid agglutination or chemical flocculation methods were employed for the concentration of tubercle bacilli. The experiments were devoted to a careful quantitative analysis of the efficiency of the methods rather than to the collection of information from a long series of clinical specimens.

The procedure recommended for the concentration of tubercle bacilli from sputum will be outlined and discussed and then data will be given concerning the efficiency and usefulness of the various flocculation methods which were studied.

METHODS

Method for the Concentration of Sputum—The following reagents are required:

(a) Digestor—4 per cent sodium hydroxide which contains 0.2 per cent potassium alum and 0.002 per cent bromthymol blue.

(b) Hydrochloric acid—approximately 2.5 N (25 per cent of conc. HCl by volume).

(c) Ferric chloride solution (1 per cent of FeCl_3 in distilled water). The concentration is carried out as follows:

1. Mix 5 c.c. of sputum with an equal volume of the digestor. Digest in a water bath at 37°C for thirty minutes with occasional shaking.

2. Add 2.5 N HCl drop by drop with shaking, until the color of the indicator denotes approximate neutrality. Shake for thirty seconds. If flocculation does not occur in less than five minutes, add 0.2 c.c. of the ferric chloride solution and shake again (rarely necessary).

3. Centrifuge the flocculated sample for five minutes at top speed to pack the precipitate. Discard the supernatant fluid.

4. Prepare uniform smears on glass slides, dry in air, heat fix, and stain by Ziehl-Neelsen's method.

This procedure as stated can be applied routinely to all sputa and has given results which consistently excel (vide infra) those obtained by direct centrifugation of a portion of the digested sputum at 1000 $\times g$ * for one hour. However,

*1000 $\times g$ means a relative centrifugal force of 1000 times gravity. This symbol will be used to designate the centrifugal force which was used in all centrifugations.

a consideration of several of the factors which contribute to the quality of the results and information concerning certain details of the technique, will assist in employing the method to the greatest advantage.

Since it is the purpose of any concentration method to collect the largest possible number of tubercle bacilli in the smallest possible volume, it is obvious that homogenized sputa which contain an appreciable amount of undigested residue should, prior to flocculation, be centrifuged at 500xg for five minutes to remove the debris so that a bulky precipitate does not result from the flocculation. This step does not remove a significant number of bacilli and need not require extra glassware, because the alum precipitate can, if necessary, be made in the same tube and collected on top of the packed debris. In order to obtain small precipitates the concentration of alum finally chosen was the smallest amount which would flocculate in the majority of sputa. Occasional sputa inhibit the flocculation. These samples are flocculated with ferric chloride (hydrous ferric oxide) which ranks next to alum in efficiency.

Flocculation of the alum will occur over a pH range of 4.5 to 9.5. It is only necessary that the reaction be brought within the useful range of the bromthymol blue. A drop or two of the digester is added for back-titration if the sputum digest is made too acid. The pH zone of 7.0 was chosen because very bulky precipitates of protein and alum occur at lower pH ranges (especially at pH 4.7) and because films from alkaline precipitates do not stick well on the glass slides. The approximately neutral precipitates are also most suitable for cultivation or animal inoculation.

It is important that the precipitates of alum or ferric hydroxide do not require an electrical centrifuge for collection. They can be packed in a hand centrifuge, collected by passing the flocculated sample through a small pleated filter paper, or even by aspirating the fluid into a pipette which is plugged at the bottom with absorbent cotton. In the latter cases, the smears may be made by rubbing the filter paper or the cotton directly on the glass slides.

The precipitates obtained with the flocculating agents are smooth and creamy, and should not be spread by pressing them between two glass slides. They should be smeared evenly over the surface of the glass slides, using a technique similar to that employed for the preparation of Breed smears from milk. The uniformity of the films, together with the even background provided for the microscopic fields, permits the preparation of quite heavy smears and thus facilitates the microscopic examination.

Method for Flocculation in Urine.—To each 10 c.c. of the urine, add 0.5 c.c. of 5 per cent alum solution, or 0.5 c.c. of the 1 per cent FeCl_3 solution and, if necessary, adjust the pH reaction to the neutral range. The sample should be shaken violently at the moment that the agent is added. An occasional urine will inhibit the flocculation of these agents because of containing unusual amounts of urea or other substances which are known to prevent the precipitation of these hydrous colloids. In such cases, more of the chosen reagent should be added, and the shaking of the system should be delayed until considerable

precipitation of the newly added reagent is observed. The precipitate should then be dispersed throughout the urine by violent shaking. This modification of the precipitation method is in reality similar to the use of freshly prepared creams of alum or non (vide infra).

EXPERIMENTAL DATA

Comparison of Various Flocculation Methods—At first two flocculation methods were compared with Petricoff's method of concentration by direct centrifugation. After the superiority of the two flocculation methods had been established, these methods were compared with other flocculating procedures which were designed to simplify the technique and to improve the degree of concentration.

Since the methods were changed repeatedly, only the general scheme of procedure can be outlined as applying to all experiments.

The sputa were collected* in sterile glass bottles which contained a few glass beads. The samples were homogenized on the same day by violent shaking or by digestion for thirty minutes in a water bath at 37° C with an equal volume of acid or alkali. When uniform portions of the same digest were desired, the homogenized sputa were poured into sterile 50 cc centrifuge tubes and centrifuged at 500 $\times g$ for five minutes to remove any debris. This step did not cause sedimentation of the bacilli and served to provide an absolutely uniform fluid on which to test the various methods of collection. Five cubic centimeters of the homogeneous digest were placed in each of the required number of 15 by 120 mm round bottomed Pyrex tubes and subjected to the desired method of concentration.

Following concentration the supernatant fluids were poured off and the tubes were drained for five minutes while inverted on paper towels which had been soaked in $HgCl_2$ (1:1000). The sediment was taken up in serologic pipettes (graduated to 0.01 cc) and 0.01 cc portions were carefully spread over marked areas of 20 sq cm on clean glass slides in duplicate.

The films were fixed by heat and stained at 37° C for one hour in a Coplin jar which contained carbol-fuchsin. The stain was poured off, the slides were rinsed in water and decolorized with alcohol containing 3 per cent by volume of concentrated hydrochloric acid. They were counterstained with methylene blue for one minute. During examination of the slides both the number of acid fast bacilli seen and the number of fields counted were recorded. Since the occurrence of the bacilli in clumps does not increase the possibility of discovering the organisms in stained smears, all clumps were recorded as a single bacillus.

The first series of quantitative tests on positive sputa were made to compare the number of bacilli which could be collected from 5 cc portions of the same NaOH digests by (a) centrifugation at 1000 $\times g$ for sixty minutes (Petricoff's method with prolonged centrifugation), (b) centrifugation at 1000 $\times g$

*We are indebted to Dr. A. C. Leonardo of the Tuberculosis Hospital of Washington, D. C. who very kindly furnished the sputum and information concerning the positiveness or negativeness of sputum from each patient on the basis of repeated direct microscopic examination.

for sixty minutes after the H-ion reaction of the sputum was adjusted to the isoelectric point of tubercle bacilli (pH 2.8 to 3.0), (c) centrifugation for three minutes of alum precipitated, and (d) of iron precipitated samples (see methods in the next paragraph). The results showed that although the isoelectric method permitted the collection of more bacilli than plain centrifuging of the NaOH digests, it was definitely inferior to alum or iron flocculation in the collection of the bacilli. The isoelectric method was not studied further.

A second series of tests was carried out in which homogeneous NaOH sputum digests were employed in determining the number of bacilli which could be found by examination of the following: (a) the neutralized digest, (b) sediment from centrifugation, (c) alum precipitate, and (d) iron precipitate. The alum flocculations at that time were made by adding 0.5 c.c. of 5 per cent alum to each 5 c.c. sample (final concentration of 0.5 per cent alum), followed by the addition of 2.5 N HCl to produce a pH of approximately 4.7 in the presence of bromeresol green indicator. The iron flocculations were made by adjusting the pH of the sample to a pink color of phenolphthalein and then adding 0.25 c.c. of 1 per cent FeCl_3 solution (final concentration of 0.05 per cent), which caused precipitation at a pH of approximate neutrality.

TABLE I
COLLECTION OF BACILLI FROM A KNOWN POSITIVE SPUTUM

METHOD	CENTRIFUGING TIME IN MINUTES*	BACILLI FOUND IN 50 FIELDS	CONCENTRA- TION FACTOR
(a) Direct examination of sputum digest	—	9	x1
(b) Centrifugation	60	64	x7
(c) Alum flocculation	3	127	x14
(d) Iron flocculation	3	169	x19

*Centrifugation at 1000xg.

Table I shows typical results from the concentration of a positive sputum. The digested sputum, rather than the original, was used in the direct microscopic examination, because it appeared the only means of obtaining a sufficiently even distribution of the bacilli to make the counts significant. The increased numbers of bacilli seen as the result of concentration are expressed as a "concentration factor" which is based on the direct examination as unity.

The table indicates that the concentration methods increased the number of bacteria in fifty fields by factors of 7 (direct centrifugation), 14 (alum), and 19 (iron). It must be remembered, however, that the digestion process doubles the volume of the sputum sample. Unless the process of homogenization breaks up clumps of bacilli sufficiently to double the possibility of finding them on direct examination, these concentration factors must be halved in order to indicate the extent to which the concentration methods actually enhance the possibility of demonstrating bacilli in a tuberculous sputum.

Table II shows the results of applying the foregoing concentration methods (as described) to 8 sputa which were known to be repeatedly negative on the basis of direct microscopic examination at the Tuberculosis Hospital. In our hands the examination of 200 microscopic fields gave negative results on the

unconcentrated samples. It will be noted that after concentration all of the sputa remained negative (100 fields) with Petroff's method and with the alum flocculation method, while two sputa were positive in the ferric chloride (non) concentrates.

TABLE II

RESULTS ON EIGHT SPUTA WHICH WERE REPORTEDLY NEGATIVE BY DIRECT MICROSCOPIC EXAMINATION

Each sputum was digested with 4 per cent NaOH and given a preliminary centrifugation. The homogeneous supernatant fluid was then concentrated by three methods: (a) direct centrifugation, (b) alum, and (c) non (ferric chloride) flocculation.

SPUTUM NUMBER	Bacilli found in 100 microscopic fields following			Results of cultivation,† indicating the number of culture tubes which were positive for each concentration method		
	CENTRIFUGATION* 45'	ALUM* 3'	IRON* 3	CENTRIFUGATION* 45'	ALUM 3'	IRON 3'
1	—	—	—	—	—	—
2	—	—	—	—	—	—
3	—	—	7	2	4	1
4	—	—	—	—	—	—
5	—	—	—	—	—	—
6	—	—	—	—	—	—
7	—	—	2	0	2	1
8	—	—	—	—	—	—

*Centrifugation at 1000 $\times g$ for the time indicated (' = minutes)

†The sediment from each concentration method was cultivated on 6 tubes of medium

At the same time the sediment from each concentration method was seeded on Cooper and Urey's glycerol potato medium.¹ It is of interest that positive cultivation results occurred only in the two sputa which gave positive microscopic results, and that the alum precipitates gave a higher incidence of growth than the other sediments.

Although there has not been opportunity for further study of cultivation from sputum by the use of flocculated sediments, it has been ascertained (unpublished data) that the alum and non precipitates do not interfere with the growth of minimal numbers of tubercle bacilli or other bacteria.

Simplification of the flocculating procedures was undertaken by (a) the preparation of "creams" of alum or non which could be stored and added to specimens just prior to their centrifugation, and (b) by the incorporation of the flocculating agents in a "digestor" so that flocculation would occur on neutralization of the digested sputum.

Alum or Iron "Creams"—The 1 per cent FeCl_3 solution was precipitated by the addition of $\text{N}/10 \text{ Na}_2\text{CO}_3$ in the presence of bromthymol blue indicator, until the supernatant fluid above the settling precipitate showed a pH reaction of approximately neutral. The alum "creams" were prepared by the addition of $\text{N}/10 \text{ Na}_2\text{CO}_3$ to a 5 per cent solution of alum in the presence of bromocresol green indicator and adjusting the pH to approximately 4.7.

The alum and non precipitates were then adjusted in volume so that 1 cc of the suspension was required for each 9 cc of sputum sample. These

"creams" were tested for their collection powers while fresh and also after autoclaving to hasten the aging process which is known to occur in all such hydrous colloids. The dispersion of the fresh, and especially the heated (aged), creams in digested and neutralized sputum samples gave results which were somewhat inferior to those which were obtained by making the flocculations directly in the individual samples. These "creams" were felt to be of the greatest usefulness only in concentrating specimens such as urines which inhibit the formation of precipitates.

Incorporation of the Flocculating Agent in the Digestor.—The incorporation of ferrie chloride in acids or in NaOH is not feasible. This salt is insoluble in alkali. It did not precipitate on neutralization of oxalic or sulphuric acid digests of sputum because its flocculation was inhibited by the presence of proteins in the sputum. Although 1 mg. of FeCl_3 per cubic centimeter of acid digester precipitates on neutralization in aqueous systems, 1 per cent of egg albumen completely inhibits the flocculation of many times as much ferrie chloride.

The alum is soluble in either acid or alkaline digestors, and final concentrations as low as 0.05 per cent alum flocculate rapidly in water when neutralized to the pH range of 4.5 to 9.5. Two-tenths per cent alum and 0.002 per cent bromeresol green were incorporated in 4 per cent NaOH and in 6 per cent H_2SO_4 to prepare alkaline and acid "digestors" which would flocculate in the presence of an equal volume of sputum when neutralized to pH 4.7.

The ten sputa in the next series of tests were well shaken with glass beads prior to the removal of three 2.5 c.c. samples which were concentrated as follows: (a) by NaOH digestion and subsequent iron flocculation (best method hitherto), (b) by digestion with 4 per cent NaOH containing bromeresol green color indicator and 0.1 per cent of alum, and (c) by digestion with 6 per cent H_2SO_4 containing the color indicator and 0.2 per cent of alum. In the last two cases the digested sputum was merely adjusted to a pH of approximately 4.7 in order to induce the flocculation. The bulk of undigested debris in the acid digester was so great that all of the samples done by this method required a preliminary centrifugation before flocculation of the alum. After 100 fields had been counted in the films from each sediment from each of ten sputa, the methods were rated in accordance with the number of times each had collected the highest number of acid-fast bacilli. The ratings were as follows: iron flocculation—5, H_2SO_4 -alum—3, and NaOH-alum—1; one sputum was negative by all three methods.

These tests were the first in which any alum method gave better results than the iron method in part of the tests. However, the relatively good results with the alum in an acid digester must be attributed in part to the fact that all the debris had been removed from these samples by a preliminary centrifugation while only one or two of the alkaline digests were cleared of debris in the same way. The constant occurrence of large amounts of undigested residue in the acid digestors interfered with the quality of the results unless the debris was removed. This inconvenience was regarded as sufficient to disqualify the H_2SO_4 -alum for further study.

The NaOH alum digestor was as effective in homogenizing and clarifying sputum as the plain NaOH digestor, and was continued in the hope of reducing the bulky precipitates which alum had always produced at pH 4.7, even from very clear sputum digests.

A solution of this difficulty followed the discovery that the bulky flocculations with alum at pH 4.7 were largely due to precipitation of the proteins from the sputum at that pH. New NaOH alum digestors were prepared to contain varying amounts of alum with 0.002 per cent bromthymol blue as the color indicator. It was learned that at least 0.2 per cent of alum must be used in order to cause flocculation in the majority of sputa when the final pH is adjusted to approximately neutral.

A new series of 14 sputum samples was then concentrated for the purpose of comparing the results of flocculation of the NaOH alum digestor at a pH of approximately 7.0 with the results of the established ferric chloride method. In this series the final concentration of FeCl_3 was reduced to 0.02 per cent because it had been learned in the meantime that this amount of FeCl_3 produced higher counts than the 0.05 per cent which had been used hitherto.

TABLE III

COMPARISON OF THE NaOH ALUM FLOCCULATION AT PH 7 AND THE ION FLOCCULATION OF NaOH DIGESTS

The sputa were shaken with glass beads before removal of 5 cc samples to be concentrated by each method. After flocculation, the precipitates were collected by centrifugation for five minutes.

SPUTUM		METHODS			
		Addition of 0.1 cc of 1% FeCl_3 to 5 cc of NaOH digest		Neutralization of NaOH alum digest to pH 7.0	
	Direct examination of 200 fields	Fields	Bacilli	Fields	Bacilli
1	Positive	100	38	100	110
2	Positive	50	50	50	120
3	Negative	200	0	200	0
4	Negative	200	0	200	5
5	Negative	200	17	200	44
6	Negative	200	0	200	6
7	Negative	200	2	200	3
8	Positive	50	97	50	126
9	Negative	100	0	100	5
10	Negative	100	0	100	7
11	Negative	200	0	200	0
12	Negative	100	0	100	2
13	Negative	100	15	100	21
14	Negative	100	0	100	3

The results as shown in Table III indicate that whereas 11 sputa were negative by direct examination only 2 were negative following flocculation with alum. The alum flocculations at pH 7.0 were consistently superior to the non method. The improvement of the alum method was attributed to the fact that it no longer gave bulky precipitates. One sputum (No. 3) however inhibited flocculation with the amount of alum employed and required precipitation with ferric chloride.

A more detailed consideration of the results obtained with sputum number 8 is interesting, because the degree of concentration achieved by the flocculation

methods was again compared quantitatively against the microscopie counts of the unconcentrated sputum digest. The unconcentrated sputum digest gave average counts of 2.5 bacilli per 50 fields, which gives a concentration factor of approximately 50 times for the alum method and of 40 times for the ferrie chloride method. These results are much better than those obtained in the first series of tests on alum and iron flocculation (see Table I, for example).

DISCUSSION

We have found that other investigators have used the flocculation principle in collecting tubercle bacilli from sputum or urine. Professor W. A. Starin, of Ohio State University, has used alum from time to time since 1909 in making concentrations from sputum and urine. Although we do not know the procedure employed or the exact degree of concentration obtained, Professor Starin reports² that 8 to 15 per cent more positive results were obtained with alum than with other concentration methods. Ditthorn and Schultz³ concentrated alkaline sputum digests by the addition of 5 per cent by volume of a 20 per cent ferrie oxychloride solution and recovered the precipitates by suction filtration. Among 60 sputa which were negative by direct examination and by antiformin concentration, three were shown to be positive by precipitation. Pfeiffer and Robitseeck⁴ collected the bacilli from digested sputum by adding 2 c.c. of mastie solution in alcohol to 8 c.c. of sputum and allowing the precipitate to stand over night before centrifuging. Saellhof⁵ has reported trials with alumina creams as collecting agents and found a slight increase in the number of bacilli collected. Petroff⁶ has used tannic acid in precipitating tubercle bacilli from urine for some time and has obtained better results than by direct centrifugation.

All these investigators have shown that by flocculation methods it is possible to obtain more bacilli than by direct centrifugation. It is unfortunate that the great inefficiency of centrifugation as a collection method has not been realized to such an extent that the methods might have been more fully developed.

The physical mechanism of the flocculation of tubercle bacilli has not been investigated. It is assumed tentatively that the negatively charged bacilli are collected (not adsorbed) by the positively charged precipitates. On this basis, the somewhat less efficient collection of bacilli from sputum by the use of the prepared "creams" could be explained on the hypothesis that the particles of precipitate were not dispersed in a sufficiently fine state or for a sufficient time to come in contact with all the bacilli. In the iron method, the ferrie chloride is precipitated instantaneously on being mixed in the neutral or alkaline sputum digest. Although the samples were shaken as quickly as the pipette could be withdrawn, it is possible that the iron flocculations depended largely on collecting the bacilli with a very recently formed precipitate.

On the other hand, in the precipitation of an agent such as alum, which has been dissolved in the system (NaOH-alum method), the flocculation is necessarily initiated throughout the entire sputum digest and more complete collec-

tion of the bacilli should take place. This reasoning is supported by the fact that the highest yields of tubercle bacilli were obtained by neutralization of the NaOH alum digests at a pH which gave precipitates as small as those produced with the non flocculation (see Table III).

The undesirable debris which occurs in any acid method of digestion definitely interferes with the usefulness of acid digestors for microscopic work [see also Pinner], but is probably not a disadvantage in cultivation. It has been shown that the removal of debris from either acid or alkaline digests by a preliminary slow centrifugation increases the number of bacilli per microscopic field, irrespective of the concentration method employed. Therefore, preliminary centrifugation of the digests is as important to good results by direct centrifugation as it is by the flocculation methods.

The fact that the amount of alum chosen occasionally fails to flocculate in a sputum is not regarded as a serious disadvantage in view of the fact that the addition of ferric chloride will always induce prompt flocculation in such sputa. It must be remembered that physical factors such as the specific gravity and viscosity of different sputa have always made the collection of tubercle bacilli by direct centrifugation a procedure of variable reliability as well as of low efficiency. It is believed that the flocculation methods not only increase the efficiency of collection but also minimize the effect of variations in the density and viscosity of the sputum digests.

CONCLUSIONS

Methods have been developed for improving the collection of tubercle bacilli from sputum and other body fluids. The recommended procedure involves the incorporation of alum in the NaOH used for digesting the sputum samples. When the sputum digest is neutralized the alum flocculates and collects the tubercle bacilli. This method is as simple as any of the present methods of concentration and possesses the following advantages:

- (a) It reduces the centrifugation time to five minutes or, if filtration through paper is desired, dispenses with centrifuging entirely.
- (b) It permits the preparation of very uniform and rather thick films thus facilitating the microscopic examination.
- (c) It collects the bacilli more completely from the sputum or other specimen so that a unit amount of sediment contains from 3 to 7 times more bacilli than can be collected by direct centrifugation of the same sample.

In addition, it has been shown that the flocculated precipitates do not interfere with the cultivation of the tubercle bacilli when very small numbers of organisms are present.

It has been learned that extremely thick viscous sputa require more than one volume of digester. Personal communications from several investigators have indicated that as much as five volumes of plain NaOH must be used in digesting such sputa in order that the bacilli may be collected by direct centrifugation. In the use of alum NaOH or plain NaOH it would be desirable to have an objective means of determining the amount of additional digester which is required.

In the alum NaOH method the alternate use of ferric chloride on the rare occasions when flocculation fails assists in overcoming these difficulties.

laboratory to have reached a stable pH (about 6.3). If the pH of the various dilutions are now taken, they will be found to be 7.1, with a slight tendency to drop down in the lower concentrations. These fluids are stable for several weeks, but are inclined after some time to become alkaline, probably as a result of the effect of the glass container. When any appreciable alteration (0.1) in the pH above 7.1 has taken place, the fluid should be discarded and fresh solutions prepared.

To carry out the test, eleven of the special tubes used in the Evelyn photoelectric colorimeter are required. Ten of these are set up in a rack and into each in order is placed 20 c.c. of the various dilutions of Simmel's fluid, ranging from 100 to 30 per cent. To each of these is added 50 c.mm. of fresh blood, obtained by venous puncture and kept from clotting by the use of a minimum amount (2 mg. per c.c.) of a mixture of ammonium (6 parts) and potassium (4 parts) oxalates. The tubes are then gently shaken to insure an even distribution of blood in the fluid, care being taken, as in all photoelectric work, not to soil by handling the lower portion of the tube as this comes into the path of the light rays. At this point one may make a macroscopic determination by holding the series of tubes to the light and noting the relative amount of hemolysis and turbidity in the different solutions (Fig. 1). Any appreciable deviation from the normal can quite readily be detected in this manner after a little experience. The tubes are then centrifugalized at only sufficiently high speed to throw down the unhemolyzed erythrocytes in ten minutes.

During this interval a determination of the total amount of hemoglobin in the blood is carried out. This consists in adding 50 c.mm. of the blood to 20 c.c. of an aqueous solution of 0.4 per cent NH_4OH in the remaining tube, and after taking the reading, determining the percentage of hemoglobin from the chart, supplied with the instrument.

After centrifugalization (Fig. 2) is completed, similar readings are taken on each of the ten tubes containing the various strengths of Simmel's fluid, and the amount of hemoglobin liberated in each dilution determined from the chart. In the vast majority of cases it will be found that hemolysis is complete in the lowest, that is the "30" tube, and the amount of hemoglobin will be the same as the total amount found by using the ammonia solution; while in the higher concentrations, as the "70" and "100" tubes, no hemolysis will have occurred.

INDEX OF HEMOLYSIS

In order to correct for any anemia which may be present, it is necessary now to compare the amount of hemoglobin in each of the tubes of Simmel's fluid with the total amount as determined by using the ammonia solution and estimate the percentage of hemolysis which has taken place. That is, in a blood having 80 per cent hemoglobin, a tube showing 60 per cent hemoglobin would have 75 per cent hemolysis. In this way a series of ten figures are obtained, varying usually from 0 in the higher tubes to 100 per cent in the lowest. These represent the per cent of hemolysis in each tube. A curve (hemolygram) may be worked

out quite readily which makes obvious the finer details of the type of hemolysis and this distinguishes between cases in which practically all the cells hemolyze at one point (isohemolysis) and others in which the points of hemolysis are spread through several solutions (anisohemolysis). Also other finer degrees of difference are readily shown as in many hypochromic anemias, where there is a tendency to an abnormal resistance in the lower tubes and an abnormal fragility in the higher concentrations.

We have found it most convenient, particularly from the standpoint of reporting, to use an index of hemolysis. This is obtained by adding together the ten figures which represent the percentages of hemolysis in each of the tubes containing the Simmel's fluid (Table I). In this way a theoretical range from

TABLE I
ERYTHROCYTE FRAGILITY TEST

Example of detailed report of erythrocyte fragility test showing method of calculating index of hemolysis Normal values

NAME—CU	DATE—MARCH 15, 1937	WARD—F
TOTAL HEMOGLOBIN = 87% (13.6 gm./100 cc)		
TUBE	HEMOGLOBIN %	HEMOLYSIS %
100	0	0
70	0	0
65	0	0
60	0	0
55	6	7
50	17	19
45	74	85
40	84	97
35	86	99
30	87	100
Index of Hemolysis =		407

0 to 1000 is obtained. The former where no hemolysis takes place in any of the ten tubes, the latter where hemolysis is complete in all. The use of this index is a distinct advantage over the old methods of reporting results of fragility test and allows for the noting of very small variations which were not possible before. It does have the disadvantage that it does not allow for expressing the changes in the character of the curve of hemolysis. Where this is abnormal, however, it may be readily reported in addition to the index.

NORMAL VALUES

To determine the range of normal values for the index, it was decided to repeat, using the new method, the work carried out by Waugh and Chase with the old technique. They examined the blood of fifty apparently healthy adult males and found a considerable range of normality. This amounted to a shift of about one tube above and below a medial point. These findings were confirmed, as shown by the accompanying chart (Fig. 3). The total average index of hemolysis of the fifty males was 406. Of these the ten lowest averaged 352, the ten highest, 464, while the thirty intermediates gave an average figure

of 405. The lowest encountered in the series was 319, and the highest 542. It would appear, therefore, for the practical purposes of interpretation, from 350 to 450 should be considered within normal limits, while values above and below these figures should be reported as very slightly, slightly, moderately or markedly increased, or diminished as the case may be.

PATHOLOGIC VALUES

Under abnormal conditions the range is very great. Figures below three hundred are not uncommon in obstructive jaundice. The lowest which we have met with so far is 239 in a case of carcinoma of the head of the pancreas.

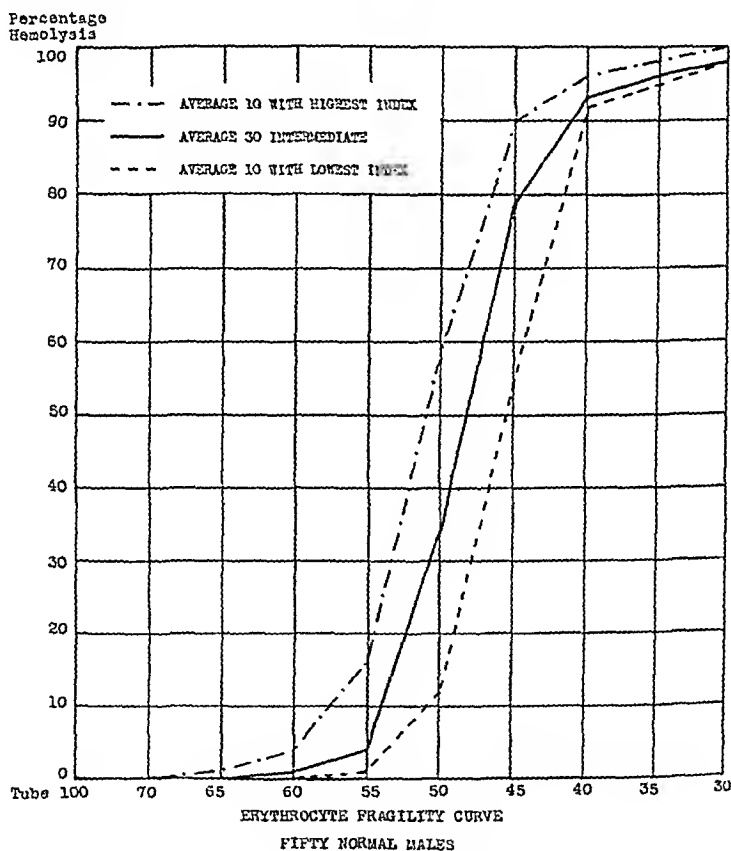


Fig. 3.—Hemolygram showing averages of findings with the fifty individuals separated into three groups.

In the hereditary type of hemolytic jaundice the index tends to run well over six hundred. Our highest figure is 674, but very much higher values probably do occur.

Changes in the fragility in the same individual are readily recorded by this method. For example, we recently noted in a case of acquired hemolytic jaundice a drop from 539 to 250 after splenectomy. While these changes have been recognized for many years, they are undoubtedly made much more vivid by the use of the index. Moreover, minor changes associated with certain age

periods as at birth or during the course of various diseases can be studied and recorded in a much more satisfactory manner

SUMMARY

A technique for determining the fragility of the erythrocytes by means of the Evelyn photoelectric colorimeter is fully described

The use of an index of hemolysis is advocated as it offers a much readier and more precise means of expressing and recording the fragility of the red blood cells

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A METHOD FOR THE COLLECTION OF PERIPHERAL BLOOD SAMPLES

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THE determination of the volume of packed red blood cells in conjunction with red blood cell counts and hemoglobin determinations is of undoubted value in the differential diagnosis of various types of anemia. Most of the hematocrit methods require quantities of blood which can be obtained only by venipuncture. The difficulties which are sometimes encountered in securing blood from the vein particularly in infants and small children have led to the development of a number of methods which make use of peripheral blood. Some of these have proved unsatisfactory, because rubber sealing devices are used on the hematocrit tubes. Slight leakage is a frequent source of error in such methods. Still others require special capillary tubes and centrifuge equipment. Smith¹ has recently introduced a new tube designed primarily for a sedimentation technique on small amounts of blood. It serves also as a very simple and accurate hematocrit tube. Certain modifications of his method have been found necessary in order that the blood sample may be used for determinations of packed cell volume, number of red blood cells, and quantity of hemoglobin in addition to the sedimentation rate.

1 Heparin seems to be the most satisfactory anticoagulant for cell volume determinations. In the small amounts required for this method it is not expensive. We have confirmed Smith's observation on its use in the sedimentation

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test; in these short 50 mm. tubes, the amount of sedimentation of heparinized blood cells in apparently healthy persons may sometimes equal that in pathologic conditions. A mixture of dry ammonium and potassium oxalate, which was suggested by Smith as a substitute for heparin, does not, in our experience, always prevent the formation of small clots.

2. The collection of blood from the finger tip or heel directly into small vials minimizes the danger of clotting. The sample can be obtained rapidly, carried to the laboratory, and examined at leisure. Greater accuracy can be secured by several check determinations of the red blood cell count and the hemoglobin.

METHOD

Vials are prepared essentially as described by Guest and Siler.² Three drops of a freshly prepared 1 per cent solution of heparin are run into flat-bottomed, wide mouth vials with a capacity of about 1 c.c. A thin film of antieoagulant is then deposited on the sides and bottom of the vials by evaporation in an oven at 100° C. The stoppers are ordinary corks which have been coated with paraffin.

The finger or heel should be warm and of good color. If it is cyanotic and cold, the extremity should be immersed in water at 38 to 40° C. for 3 to 5 minutes to reestablish active circulation. After washing with alcohol, a thin film of sterile vaseline is applied to the site chosen for puncture. The vaseline facilitates the formation of well-rounded drops of blood and helps to prevent clotting. It may also be applied around the mouth of the vial if the blood tends to adhere to the glass. A puncture wound deep enough to give a free flow of blood is made with a spring lancet, and approximately 0.5 c.c. of blood is collected. The vial should be shaken occasionally during collection to prevent clotting.

The Smith tube is filled with blood to the zero mark by means of a capillary pipette and rubber bulb. It is allowed to stand in a vertical position for one hour to determine the amount of sedimentation. The tube is then centrifuged until there is no further change in volume of the packed cells. (In an International centrifuge, type S B, 30 to 45 minutes at about 3,000 r.p.m. is sufficient.) Red blood cell counts and hemoglobin determinations are made on the portion of the sample which remains. When all these values have been obtained, the average volume and concentration of hemoglobin in the corpuscles can be determined. This method has been used to follow the red blood cell values of a group of infants and small children for the past year and has proved entirely satisfactory.

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COLOR PHOTOGRAPHY AND ITS APPLICATION IN MEDICAL TEACHING*

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ROUTINE color photography has in our experience simplified teaching and brought about a new era for the department of pathology. It gives renewed interest to the laboratory workers, encourages teaching, and supplies us with a method of collecting material for demonstrations which will be invaluable in time to come. It is our practice to make color photographs of all material of teaching value which comes to our department. Furthermore, we frequently anticipate interesting autopsy material and make ante mortem color pictures of the patient in order that they may be filed with the postmortem pictures.

The value of color pictures in medical legal aspects of medicine cannot be over emphasized. Recently a woman and her husband were both found twenty four to thirty six hours after death. Examination revealed evidence that the man had killed his wife and later shot himself. The death grip of his hand on the revolver left certain very important evidences which were preserved most satisfactorily by color photography.

Each exposure or transparency is mounted between cover glasses to make lantern slides, 50 by 50 mm. numbered and filed in a numerical manner corresponding to the system carried out in connection with other specimens and reports in the laboratory. These are indexed and cross indexed and in case of autopsy material and gross specimens the notation of "photograph made" is included in the laboratory report. The actual cost of materials averages twenty cents per slide, which includes developing.

The lighting for making transparencies in color should be flat. The necessity of this is to be amplified when it is recalled that in color photography good contrast is obtained almost entirely from the colors of the object, bright highlights and deep shadows are to be avoided. This is accomplished by arranging the lights close to the camera and directing them upon the object in such a manner that equal distribution of the illumination is obtained.

We are using Kodachrome film of which there are two types, the regular for use in daylight, and the Type A which is used only with artificial light. The Type A has a Weston speed of 8 and, being less sensitive to red light, is used without filters.

A miniature camera constructed to use standard 35 mm. motion picture film may be used satisfactorily. The well known Leica camera has been used

*From the Pathology Department, University Hospital, University of Oklahoma School of Medicine.

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entirely in making color transparencies in our laboratory. These are precision-made cameras of high quality and supplied with excellent optical equipment of microscopic accuracy. They are remarkable for the ease and simplicity of operation, and their universal adaptation to any photographic need. They are equipped with high speed lenses which permit short exposures under unfavorable lighting conditions. The short focal length lenses permit great depth of focus at short range, a necessity in close-up photography. Models with slow shutter speeds up to one full second are of distinct advantage.

The sunshade should always be used over the lens to eliminate stray light. Strong light is desirable to make possible a short exposure, also to permit "stopping down" the iris diaphragm. Two No. 1 photoflood bulbs in each of two reflectors are used routinely. The intensity of the light is always measured with a Weston photoelectric cell. This is quite important and must be emphasized in view of the fact that correct exposure is essential in obtaining good color rendition. Over exposure of Kodachrome results in color transparencies which are too pale, thin, and lacking in color quality. Underexposure results in transparencies that are dense and dark. In making the light intensity readings it is essential to place the meter close to the object to be photographed, but not in its own shadow. In deeply colored objects, a reading should be taken on a white sheet, towel, gauze, or paper to determine maximum intensity and an average of these taken. A slight underexposure is the usual aim.

For near objects a short optical focusing device is available which permits automatic focusing on all objects from $3\frac{1}{2}$ feet to 17 inches. Within this range, the head, shoulders, arms, and chest may be easily included in the field of view. Focusing is accomplished quickly by rotating the device in which the lens is fastened. It is not necessary to use a tripod or an upright arm, and the exposure can be made rapidly and conveniently with the camera held in the hand. This saves much time and is a decided advantage in photographing surgical procedures at close range. For specimens of small size and for magnifying certain portions of others and for copying charts, pictures or printed matter, a sliding copying attachment should be used. This device contains a ground glass focusing screen above the camera lens, and the camera simply slides into position over the lens and the photograph is made. Extension tubes, which place the lens farther from the camera, give greater magnification.

Gross specimens to be photographed are washed free from blood, and the moisture blotted up with a cloth to avoid glaring spots or high lights as much as possible. We have used a polarizing filter over the camera lens with some success in eliminating these and with a decided improvement of color in many of the specimens. When using a polarizing filter, it is necessary to view the object through the filter with the lights on and rotating either right or left until the glaring surface fades or the field appears darkest. In this position the filter is slipped over the lens and the photograph made. The exposure time must be increased 50 per cent. Our experience in the photography of specimens in color under water has been discouraging, since it is almost impossible to make the exposure before the water again becomes bloody, and if all the blood is washed from the specimen, the color will have faded. A

reddish tinge to the water results in very unpleasant appearing transparencies. It is desirable to use a white background for this work. Large pieces of fine mesh gauze or a white towel make a nice background when the camera is not closer than two feet. A frosted plate glass coated with white enamel on the opposite side makes a most satisfactory background. It may be quickly washed free of blood or other contamination without changing or molesting the arrangement of the specimen. Portions of the specimen should not extend beyond the borders of the field unless the purpose is to magnify small areas of it. When several specimens are to be photographed together, the arrangement should correspond to their normal anatomical position in the body. It is better to photograph specimens separately, especially when some definite or particular type of lesion is to be emphasized.

In addition to freedom from vibration and a satisfactory light source, the optical equipment must be of the highest quality in making photomicrographs in color. The camera must be one adapted to use 35 mm perforated film when using Kodachrome for this work. Any standard make of microscope may be used, but the heavier research models are better because of the greater freedom from vibrations, and by means of the additional adjustments, the optical axis may be kept more perfectly in alignment. Apochromatic objectives are essential equipment, and since color filters are not used with Kodachrome Type A film, the microscope optics must be of the highest chromatic correction. An aplanatic achromatic substage condenser is also necessary since the latter gives a color free range of light. Periplane eyepieces are used with apochromatic objectives. Obviously, the iris diaphragm, substage condenser, and objectives must be centered in the optical axis.

A satisfactory light source is the 108 watt, six volt ribbon filament globe in a lamp house containing an adjustable condenser. A water cell, 2 inches thick, is placed between the lamp and microscope to absorb heat which might possibly overheat the condenser and objectives of the microscope.

In making photomicrographs in color it is very essential that the specimen be well stained. Most tissues are stained with eosin and hematoxylin, and these should show good blues and reds since contrast is dependent upon the colors present and not upon color or contrast filters such as used in making black and white photomicrographs. In any case the color rendition of the photomicrograph cannot be better than those existent in the stained slide. The tissue should not be cut too thick. Those of 7 to 10 micra are most satisfactory, and No. 1 cover slips should be used. With the above light source placed from 10 to 12 inches from the microscope, the exposure for Kodachrome Type A film will be approximately as follows: 16 mm lens with 10 × eyepiece, 1/125 second, 8 mm lens with 10 × eyepiece, 1/50 second, and 4 mm lens with 10 × eyepiece, 1/25 second. It is essential to rest the eyes frequently as the intense light may result in faulty focusing. Records of the exposures should be kept since an exposure meter is not available at present for measuring light intensities through the microscope. Good results are dependent upon correct exposure and only by studying the transparencies when they have been returned after development can the technique of making good photomicro-

graphs in color be mastered. When the same arrangement of the light, cooling cell, and microscope is maintained, the results should be reasonably constant. The question of magnification sometimes arises. Although detail cannot be increased by great magnification, we find that greatly enlarged blood cells on color transparencies can be studied before x-ray view boxes. This method has proved valuable in teaching hematology to medical students. A few of these transparencies replace several demonstration microscopes in the laboratory, and no time is lost in finding certain fields and cells.

Although copies cannot be easily made of Kodachrome transparencies, good natural color prints on paper can be made by one of several methods. We have used both the Chromatone and the Ruthenberg Colorstill processes with satisfactory results. Printing in color at present is impractical because of the cost.

The pathologist and the pathology department of every hospital should assume teaching responsibilities. We believe color photography, as carried out by the simple inexpensive method described, is now practical, and furthermore, adds greatly to the facilities of teaching.

INTRADERMAL ANTUITRIN-S IN CHILDREN*

I. P. BRONSTEIN, M.D., CHICAGO, ILL.

THE announcement of a rapid and economical test¹ for pregnancy by the intradermal use of antuitrin-S derived from pregnancy urine was of sufficient interest to warrant its application to various endocrinopathies in children, including a number of cryptorchids.

Strauss,² in repeating the work of Porges and Pollaczek,³ suggested that it might be an invaluable aid in girls with irregular menses. It is established that the urine of normal children contains little or no gonadotropic hormone.⁴ Certainly, then, if the test is specific, one should obtain positive skin tests (negative pregnancy tests) in children, unless moderate amounts of gonadotropic principle are present, as is true of the first half of pregnancy, when this can be ascertained by the Aschheim-Zondek or Friedman pregnancy tests.⁵ In normal pregnancies 2,000 to 6,000 gonadotropic hormone units per liter are found, although amounts up to 50,000 units have been obtained.

The intradermal skin test, if indicative of significant amounts of gonadotropic substance, would prove a valuable adjunct, for by means of it, we could avoid the task of assaying for small amounts of antuitrin-S by the Zondek precipitation method.⁶ This method demonstrates a minimum of 66 mouse units per liter of urine. A negative reaction does not rule out the presence of

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minimal amounts of gonadotropic principle, but for practical purposes it is considered as indicating absence of the sex principle of the anterior pituitary. For, given a positive pregnancy test (negative skin test), it would not be necessary to repeat an assay for only 66 units, since this reaction already signifies considerable amounts of gonadotropic material in excess of this unitage.

The technique employed by Gillfillen and Gregg consisted of an intradermal injection of fresh antuitrin S kept in a refrigerator. The ventral surface of either forearm was used for the injection. An ordinary 2 cc hypodermic syringe with a 26 gauge needle was employed. Alcohol was not used as it reduces the potency of the product. I used exactly the same technique with the exception of a tuberculin syringe.

After introducing the 2 mmms one half hour is allowed to elapse before reading the reaction. If a slight reaction appears, one waits for another half hour before reaching a conclusion. The patient need not be observed further if no reaction occurs at the end of one hour. A number of children showed a reaction at the half hour period with a subsequent disappearance at the hour.

The reaction consists of an area of erythema at the site of injection measuring in diameter from 7.0 to 40 mm. If a bleb raised by the injection became red and measured 7 mm or less, but the skin adjacent to and surrounding the bleb did not become red, this was considered a negative reaction.

The reaction in nonpregnant women usually began immediately following the injection, or within one to three minutes. In every case observed, a patient who reacted with a positive skin test (negative pregnancy reaction) had a well defined erythema at the end of fifteen minutes, except in women of thirty or over, who gave a delayed reaction, and those nearing the menopause where the reaction took place as late as three hours.

The use of a simple and reliable pregnancy test was originally suggested by Porges and Pollaczek³ whose technique differed somewhat. They used 0.2 cc of hormone derived from the anterior lobe of the hypophysis. If the woman was pregnant a distinct areola, an inch in diameter, was formed after a few hours at the site of injection, and remained from twenty four to thirty six hours. Pregnant women gave no reaction. This phenomenon was explained as a specific skin reaction analagous, perhaps, to the Schick and Dick tests. Gillfillen and Gregg said that since the pregnant woman contained gonadotropic principle in her system she should not be sensitive to its intradermal injection as the nonpregnant one is, the latter showing a reaction to its presence.

Schneider and Cohen⁷ recently showed definitely the nonspecificity of gonadotropic factor of pregnancy urine intradermally in both men and women. Their observations would indicate that by this test 107 individuals were pregnant and 11 were not. Actually, however, there were only 21 pregnancies. My results in children attest to these conclusions.

Strauss² repeated the work of Porges and Pollaczek with antuitrin the only preparation available at this time. Later, however, he obtained the prolactin or gonadotropic factor. His work led to the conclusion that the test was neither

TABLE I

NUM- BER	SEX	AGE (YR.)	DIAGNOSIS	FIRST TEST		SUBSEQUENT TESTS; COMMENTS
				1/2 HR.	1 HR.	
1	M	5	U.C.*	0	0	Negative skin test (positive pregnancy)
2	M	12	U.C. Asthma	0	0	Negative skin test (pos. preg.). Tests repeated on 3 other occasions, negative each time as follows: after 1,000 units ant.-S—before and after receiving the second 1,000 units
3	M	9½	U.C.	0	0	Negative skin test (pos. preg.) 1/2 hr. 1 hr. 1 week after 1,000 units ant.-S 0 0 1 month after 1,000 units 6 mm. 0 After 2,000 units ant.-S 3 mm. 0
4	M	6	U.C.	0	0	Negative skin test (pos. preg.) 1/2 hr. 1 hr. After 1,000 units ant.-S 12.5 mm. 17.5 mm. After 3,000 units ant.-S 10.0 mm. 0 mm.
5	M	13	U.C.	0	0	Negative skin test (pos. preg.) 1/2 hr. 1 hr. After 1,000 units ant.-S 0 0 After 2,000 units ant.-S 0 0
6	M	11	U.C.	0	0	Negative skin test (pos. preg.) 1/2 hr. 1 hr. After 1,000 units ant.-S 0 0 After 2,000 units ant.-S 30 mm. 15 mm. After 3,000 units ant.-S 0 0
7	M	8	U.C.	8 mm.	0	Negative skin test (pos. preg.)
8	M	10	U.C.	0	0	Negative skin test (pos. preg.)
9	M	8	U.C.	15 mm.	0	Negative skin test (pos. preg.)
10	M	13	U.C. Mental deficiency	0	0	Negative skin test (pos. preg.)
11	M	9	B.C.†	6 mm.	0	Negative skin test (pos. preg.) 1/2 hr. 1 hr. After 1,000 units ant.-S 3 mm. 0 After 3,000 units ant.-S 0 0
12	M	7	B.C. Colored	0	0	Negative skin test (pos. preg.) 1/2 hr. 1 hr. After 1,000 units ant.-S 0 0
13	M	6	B.C.	5 mm.	0	Negative skin test (pos. preg.) 1/2 hr. 1 hr. After 2,000 units ant.-S 3 mm. 0
14	M	6	B.C.	0	0	Negative skin test (pos. preg.) 1/2 hr. 1 hr. After 3,000 units ant.-S 0 0
15	M	11	D.A.G.‡ B.C.	0	0	Negative skin test (pos. preg.)
16	M	12	D.A.G.	0	0	Negative skin test (pos. preg.) 1/2 hr. 1 hr. After 2,000 units ant.-S 0 0
17	M	8	D.A.G. U.C.	10 mm.	15 mm.	Positive skin test (neg. preg.) 1/2 hr. 1 hr. After 1,000 units ant.-S 25 mm. 20 mm.
18	M	9	Nephrotic	7 mm.	5 mm.	Negative skin test (pos. preg.)
19	M	15	Treated Cretin (6 years)	0	0	Negative skin test (pos. preg.)
20	M	14	Dwarfism Diabetes Coeliac	0	0	Negative skin test (pos. preg.) Zondek test less than 66 units prolan

*U.C.—Unilateral cryptorchidism

†B.C.—Bilateral cryptorchidism

‡D.A.G.—Dystrophia adiposa genitalia

Unless indicated, the boys with both unilateral and bilateral cryptorchidism were otherwise normal children.

TABLE I—CONT'D

NUM- BER	SEX	AGE (yr.)	DIAGNOSIS	FIRST TEST		SUBSEQUENT TESTS, COMMENTS
				$\frac{1}{2}$ HR.	1 HR.	
21	M	17	Hypogonadism	0	0	Negative skin test (pos. preg.) Zondek and Friedman tests negative for prolan
22	F	13	Chest case	0	0	Negative skin test (pos. preg.)
23	F	9	D A G Mental deficiency	0	10 mm.	Positive skin test (neg. preg.) $\frac{1}{2}$ hr. 1 hr. After 1,000 units ant. S 10 mm. 10 mm. Assay revealed less than 66 units
24	F	10	Thomson's Disease	10 mm.	10 mm.	Positive skin test (neg. preg.) Assay revealed less than 66 units
25	F	15	Pituitary Dwarf	15 mm.	15 mm.	Positive skin test (neg. preg.) Assay revealed less than 66 units
26	F	12	Pituitary Dwarf X ray of sella showed calcified mass	12 mm.	15 mm.	Positive skin test (neg. preg.) Presumably sex function of pituitary interfered with

definite nor reliable. At a subsequent time in a personal communication to this author, Porges admitted 18 per cent error. Deutsch,⁸ repeating this work, confirmed Strauss' conclusions.

McDonnell⁹ reasoned that this skin test might be valuable in other conditions in which prolan A is excreted in the urine as in testicular tumors. He emphasized the many difficulties attendant upon correct diagnosis of lesions of the serotum. The Aschheim-Zondek test is positive in teratomas and other highly malignant tumors,¹⁰ and absent in benign lesions of the testicles. Skin tests were done in six patients with proved teratomas of the testes, the Aschheim-Zondek was performed at the same time. The results suggested, according to this author, that the skin test may even be more reliable than the pregnancy test in the diagnosis of this disease. Controls were run on 25 patients picked at random. These patients were of all ages and the diagnosis consisted of extragenital earenomas, cryptorchidism, and other diseases. A negative pregnancy test was obtained in each instance. This is at variance with my results as well as those of Schneider and Cohen. McDonnell stated that until further investigation on a larger series of patients, nothing conclusive would be asserted.

RESULTS

Intradermal tests with pregnancy urine gonadotropic substance were performed on 21 boys and 5 girls, the ages and diagnosis being indicated in Table I.

Of the 21 boys, when the first skin test was performed, only one (Case 17—dystrophia-adiposo-genitalia with unilateral cryptorchidism) gave a positive skin reaction indicative of negative pregnancy. Patients No. 20 and No. 21 gave negative skin tests (positive pregnancy reaction), nevertheless, assay for very small amounts of gonadotropic substance by the Zondek precipitation method in both, and moderate amounts by the Friedman test in the second boy was negative.

Of the five girls, four gave positive skin reactions (negative pregnancy test) and one, a girl of thirteen, with chronic chest disease, in whom the menarche has not yet appeared, gave a negative skin test or positive pregnancy reaction. Of the four positive skin tests, assay for gonadotropic factor was done in three, and each determination revealed less than 66 units.

In the subsequent testing, it is interesting to note that in patients No. 4 and No. 6, with initial negative skin tests after the use of 1,000 units in the former and 2,000 units of antuitrin-S in the latter, the skin tests became positive, but became negative again after 3,000 units. In Cases No. 17 and No. 23, the original positive skin tests remained positive after 1,000 units of antuitrin-S.

The above results are inconsistent with the presence of antuitrin-S, since this hormone in moderate quantity should give a negative skin test. However, it is known that this substance may be eliminated rapidly from the system. After the fifth month, a slight and gradual decline in amount occurs up to parturition, when a sharp fall takes place.⁵ Friedman and Weinstein¹¹ state that their recent work on the fate in man of ingested and injected pregnancy urine gonadotropic substance permits of no general statement. In their experiments they failed to detect augmented gonadotropic excretion following oral ingestion of 8,000 to 40,000 units. Similarly, it was impossible to detect augmented excretion following intramuscular injection of 480 R.U.

CONCLUSION

Intradermal skin tests with gonadotropic substance of pregnancy urine in 26 children gave five positive skin tests or negative pregnancy reactions, and 21 negative tests or positive pregnancy reactions (Table II).

TABLE II

Children studied	26
Positive Skin Tests	
(Negative Pregnancy Reaction)	5
Negative Skin Tests	
(Positive Pregnancy Reaction) (Amounts of prolan found in these children not con- sistent with pregnancy)	21

This test is not consistent with the finding of reasonable amounts of gonadotropic hormone, as is ascertainable by either the Aschheim-Zondek or Friedman Pregnancy Tests.

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THE STERILIZATION OF SODIUM BICARBONATE SOLUTION FOR INTRAVENOUS USE IN ACIDOSIS*

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IN CERTAIN conditions of severe acidosis, intravenous administration of sodium bicarbonate solution is desirable. Many physicians have hesitated to use sodium bicarbonate solution because of the difficulties of sterilizing it. Heating the solution in open vessels causes the loss of so much carbon dioxide that there results an undesirable degree of alkalinity and considerable pressure develops in a closed vessel. The usual procedure is to dissolve sodium bicarbonate, taken from the interior of a package in an attempt to avoid excess contamination, in sterile water, and then use the resulting solution immediately without further treatment. Although this procedure is practically safe (we know of no accident due to it), it is preferable, for intravenous therapy, to use only sterile solutions whose alkalinity is approximately that of blood. Moreover, this preparation is inconvenient.

We, as well as many others have avoided the loss of carbon dioxide in sterilization by the use of sealed glass ampules. For several years all the bicarbonate solutions given intravenously at the Children's Hospital have been prepared thus. Although it has been far the most satisfactory manner in which sterile sodium bicarbonate solutions can be prepared the method has the disadvantage of inconvenience, possible danger of glass in the solution from breaking the glass ampules, relatively high cost, and the necessity of the service of a skilled glass blower.

For the last year we have satisfactorily sterilized sodium bicarbonate solution in a very simple manner. Stock 5 per cent solutions are prepared from chemically pure sodium bicarbonate in freshly distilled water. Phenolsulphonphthalein is added as an indicator,[†] and the solution is filtered through a Berlefeld filter. One hundred cubic centimeter portions are dispensed into 125 cc Pyrex bottles and carbon dioxide gas from a small cylinder is bubbled into

*From the Children's Hospital Research Foundation and the Department of Pediatrics University of Cincinnati

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[†]The most convenient source of indicator is the solution containing 6 mg per cc put up in 1 cc glass ampules for intravenous injection for kidney function tests (Johnson Wescott and Dunning) 0.2 cc of this solution per liter of bicarbonate is a satisfactory concentration

the solution until the color is a yellowish peach, saturating it and displacing air from the bottle. A thoroughly cleaned vaccine stopper with retractable flap, used to keep the pouring surface sterile, is inserted at once, and the stoppered bottle placed in the clamp shown in the photograph. A piece of cloth or paper, placed between the stopper and the metal, prevents sticking. The bottle is autoclaved and allowed to cool before removal of the clamp and then shaken to redissolve the carbon dioxide which has been driven off. Any pink color less than

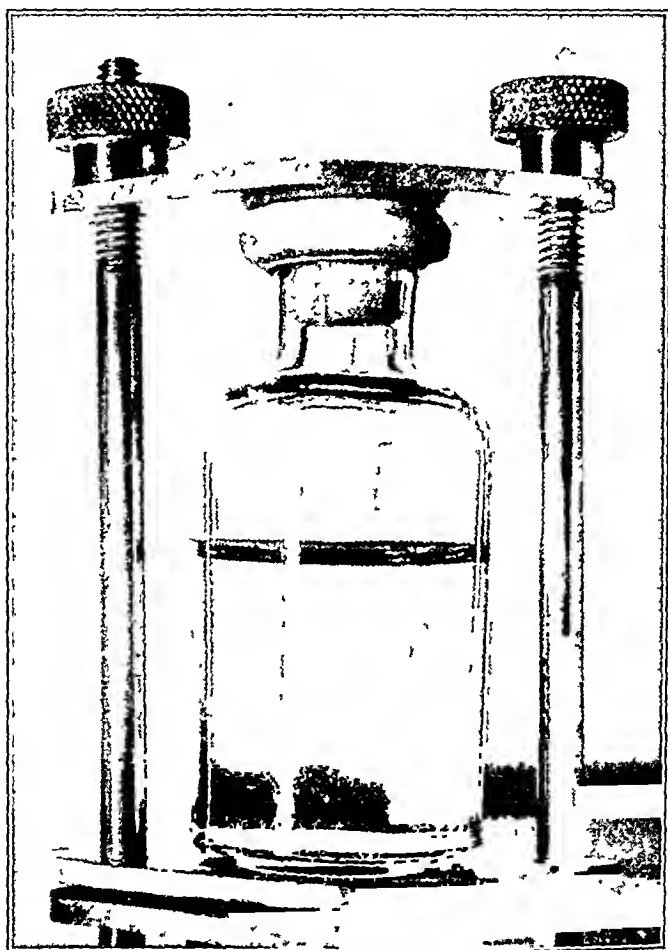


Fig 1.—Assembly for sterile bicarbonate solution. Bottle is of Pyrex glass and the stopper is the vaccine type with retractable flap. The plates of the clamp are aluminum and the rods and nuts are brass.

a deep purplish red indicates that there has been no loss of carbon dioxide from the bottle and that the pH is between 7.0 and 8.0.

In use part of the contents may be withdrawn through the vaccine stopper with a sterile needle, or the entire contents may be poured into a dispensing container for intravenous drip therapy.

When relatively large batches are prepared for hospital use, it is convenient to filter through a Berkefeld "N" filter and to use a tube with a sintered glass tip for the aeration in order to facilitate more rapid absorption of carbon dioxide.

THE REACTION OF THE SLRA OF DIFFERENT ANIMALS TO THE KAHN, KLINE, IDE, EAGLE AND LAUGHLIN TESTS*

ROBERT A. GREENE, PH D, HARRY B. HARDING, B S, WILLIAM T. HUNSPETH, B A,
AND WILLIAM J. PISTOR D V M TUCSON, ARIZ

IT IS recognized that the normal sera of various animals may give a positive Wassermann reaction,¹ and Porro² has shown that the sera of many normal animals may give positive Kahn reactions. To our knowledge, the behavior of animal blood with other flocculation tests has not been reported in the literature.

Specimens of blood were collected from various animals, the serum was separated and inactivated at 56° C for thirty minutes, with the exception of the sera to be used for the Eagle test. In this case, the necessary amount of serum was removed after twenty minutes of inactivation.

The Kahn, Kline, Ide, Eagle, and Laughlin tests were then made upon each specimen. In some cases, there was not sufficient serum to permit all of these tests, but as many were employed as the amount of serum would permit. The technique of these flocculation tests is so well known that no further reference need be made. All antigens and reagents used in these tests were purchased from biological supply houses. The Kahn and Eagle antigens were prepared by Difco Laboratories, the Kline antigen by LaMotte, the Ide by the A. S. Aloe Company, and the Laughlin Reagent by Lederle Laboratories. The results of each test were checked by at least two of us, and with the exception of the Kahn test, the results were reported only as positive, doubtful, or negative.

The results of these tests are given in Table I, and in Table II, they are given in terms of percentage. Although there was not enough sera in every case to permit the use of each of the tests, the Kahn and Kline tests were made on each specimen.

An examination of Table II reveals that all of the animal sera tested gave a relatively high percentage of positive reactions with at least one of the tests. In general, the Kline and Laughlin tests gave the highest percentage of positive reactions, and the Eagle and Ide tests gave the highest percentage of negative reactions. It should be mentioned that in some cases (particularly cow sera) the Eagle test, when positive, often did not give the sharp contrast between the clear fluids and the floccules which is characteristic of the positive reaction.

In general, there was not too good agreement between the several tests employed. In one sera, one or more of the tests might give a positive reaction and the others might give doubtful or negative results, while in another sera from the same species, the reactions given by the tests might be reversed.

*From the Arizona State Laboratory and the Department of Animal Husbandry, University of Arizona.

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DISCUSSION

These results show that the sera of normal animals give a relatively high percentage of positive reactions with the common flocculation tests used for the diagnosis of syphilis. This is particularly interesting when one considers the extremely low incidence of positive reactions given by nonsyphilitic human beings. Eagle¹ states that "anomalous Wassermann reactivity in human beings is extraordinarily rare, certainly less than one in 5,000."

These results raise several questions, which we do not attempt to answer:

- 1 Why do the sera of animals give positive reactions with flocculation tests used for the diagnosis of syphilis in human beings?
- 2 Within a single species, why should some individuals give positive reactions, while others give doubtful or negative reactions?
- 3 Why do the different tests give such widely varying results with animal sera when they usually agree extremely well when applied to human sera?

SUMMARY

The Kahn, Kline, Ide, Eagle, and Laughlin tests have been applied to the inactivated sera of the dog, rabbit, goat, horse, sheep, chicken, and cow.

The sera of these animals give a surprisingly large number of positive reactions with these tests.

Although the results given by the different tests vary greatly, in general, the Kline and Laughlin tests gave the highest percentages of positive reactions, and the Ide and Eagle gave the largest percentage of negative reactions.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TUBERCULIN TEST, Ointment Patch Test, Wolff, E., and Hurwitz, S. J. A. M. A. 109: 2042, 1937.

The agreement between the ointment patch test and the intracutaneous test with 0.1 mg. of old tuberculin in 1,075 observations was 98.2 per cent. This corresponds closely to figures previously reported for some 800 observations. Discrepancies occurred only in clinically latent cases.

The ointment test was positive in every case of active tuberculous disease.

The results of a tuberculin patch test with control herein and heretofore detailed warrant the conclusion that the test may safely be substituted for the Mantoux test with 0.1 mg. of old tuberculin in office and public health practice. The method now merits a thorough clinical trial.

UNDULANT FEVER, Diagnosis and Treatment of, Ervin, C. E., and Hunt, H. F. J. A. M. A. 109: 1966, 1937.

The results in the treatment of acute and subacute undulant fever by the intravenous injection of killed typhoid and paratyphoid organisms in carefully measured, and appropriate doses compares favorably with those of any other form of treatment.

Contraindications to its use are arteriosclerosis, arteriosclerotic and rheumatic heart disease, hypertension, and marked debility. These conditions also contraindicate most other forms of treatment.

The authors agree with Carpenter and others that the benefit comes chiefly from fever, but the stimulation of antibodies may also be important.

The advantage is the low cost and the immediate availability to any physician anywhere.

HAIR, Method of Staining Epithelial Scales and, Berberian, D. A. Arch. Dermat. & Syph. 36: 1171, 1937.

The scales are cut by means of a safety razor blade or a scalpel into small pieces, from 1 to 2 mm. square, placed on a slide and covered with a 50 per cent solution of glacial acetic acid in water. The preparation is then dried in an incubator. This process fixes the scales on the slide. The fresher the scales, the better they will adhere to the slide.

The preparation is defatted, cleared, and hydrated as follows: It is flooded with ether two or three times, depending on the thickness of the scales, the ether being kept on the slide for from twenty to thirty seconds each time. It is flooded twice with absolute acetone, the acetone being kept on the slide for from thirty to sixty seconds each time. Finally it is flooded consecutively with absolute alcohol and with 95 per cent, 70 per cent, and 50 per cent alcohol, the alcohol being kept on for about thirty to sixty seconds each time. The preparation is then stained for from three to five minutes with a solution of toluidine blue, prepared according to the formula of Martinotti which consists of 75 c.c. of distilled water, 0.5 gm. of lithium carbonate, and 1 gm. of toluidine blue.

After the dye is completely dissolved, 20 c.c. of glycerin and 5 c.c. of 95 per cent alcohol are added.

The slide is washed in water and differentiated with 0.5 per cent acetic acid

If the scales are still adherent to the slide dehydration can be best accomplished in jars containing absolute acetone. Usually three changes of acetone are sufficient to accomplish dehydration. The preparation is kept from two to three minutes in each jar of acetone and then passed through xylene and mounted in neutral balsam or preferably in euparal. If during the process of defixing or staining some of the scales have come off the slide, it is preferable to carry on dehydration with acetone by flooding the slide first with absolute acetone from three to four times and then with xylene. If no clouds appear in the xylene, it is a good indication that the preparation has been properly dehydrated. Dehydration in acetone, in contrast to dehydration in graded series of alcohols, does not remove the stain and a uniformly stained, permanent preparation is obtained. The success of the preparation depends largely on proper differentiation and proper dehydration.

PREGNANCY, Observations on the Etiology of Toxemias of, Strauss, M. B. Am J M Sc 194 772, 1937

Sodium given to 10 pregnant women with hypertension and hypoproteinemia resulted in significant weight gains, the occurrence of visible edema, and rises in the arterial blood pressure in each instance. In 5 women albuminuria increased, and in 3 symptoms of pre-eclampsia appeared.

An identical amount of sodium given to 5 pregnant women with hypertension but no hypoproteinemia resulted in small increments of weight but was without other effect.

Ammonium chloride was given to 3 and a high protein diet to 6 pregnant women with hypertension and hypoproteinemia resulted in significant weight losses and declines in arterial blood pressure.

The evidence presented suggests that the manifestations of "toxemia of pregnancy" in the patients studied resulted from water retention conditional upon hypoproteinemia.

The administration of sodium is dangerous in pregnant women with hypoproteinemia. The bicarbonate is fully as dangerous as the chloride. Its indiscriminate use in pregnancy is contraindicated.

TISSUE Silver Impregnation of Reticulum in Paraffin Sections Gomori, G. Am J Path 13 993, 1937

The author's modification is as follows:

Run paraffin sections through xylol then 2 changes of alcohol and wash under the tap.

1 Oxidize with a 0.5 to 1 per cent solution of potassium permanganate for one to two minutes. Rinse in tap water.

2 Decolorize with a 1 to 3 per cent solution of potassium metabisulfite for one minute. Wash under the tap for several minutes.

3 Sensitize in a 2 per cent solution of iron ammonium sulfate (violet crystals) in distilled water for one minute. Wash under the tap for a few minutes, then run through two changes of distilled water.

4 Impregnate with the following solution for one minute:

To a 10 per cent silver nitrate solution add one sixth to one fourth its volume of a 10 per cent solution of potassium hydroxide. Add strong ammonia water drop by drop, while shaking the container continuously until the precipitate is completely dissolved. Add again, cautiously, silver nitrate solution drop by drop until the resulting precipitate easily disappears on shaking the solution. Make up the solution with distilled water to twice its volume. It can be kept in a stoppered bottle for two days.

5 Rinse quickly in distilled water for five to ten seconds.

6 Reduce for three minutes in commercial formalin diluted with tap water to 5 to 10 times its volume. Wash under the tap for a few minutes.

7. Tone in a 0.1 to 0.2 per cent solution of gold chloride for ten minutes. Rinse in distilled water.

8. Reduce toning in a 1 to 3 per cent solution of potassium metabisulfite for one minute.

9. Fix in a 1 to 2 per cent solution of sodium thiosulfate (hyposulfite) for 1 minute.

Wash under the tap. Run through alcohol of increasing percentages. Clear in xylol and mount in balsam.

As mentioned before, paraffin sections occasionally will float away during impregnation with the strongly alkaline silver solution. This annoyance can be easily prevented by affixing the sections to the slide with gelatin instead of egg albumen-glycerin. The gelatin must be subsequently hardened by formalin fumes. The method is as follows: Dilute the glycerin-gelatin mixture commonly used for fluid preservation of sections with water or glycerin until it remains fluid at room temperature. Spread a thin layer of this solution on the slide and affix sections. Dry the slides in the incubator at 37° C. in formalin fumes for at least ten hours. (Pour commercial concentrated formalin into an open Petri dish and place it in the incubator.) The formalin has to be removed from the sections as even traces of it will inhibit impregnation. This is easily accomplished by exposing the slides in a similar manner to the action of ammonia vapor for several hours.

TISSUE: Method for Histological Examination of Long Strips of Hollow Viscera, Stein, H. B. South African J. M. Sc. 2: 117, 1937.

The method is quite simple and only requires some patience in the processes of fixation, dehydration, and embedding.

At autopsy a strip of tissue 6 to 9 inches long and $\frac{3}{4}$ inch wide is removed from the hollow viscus. The actual length of the strip in block is determined by the width of the microtome arms supporting the razor and also by the range of swing of the block holder in the microtome head. In this department a Spencer rotary microtome was found to cope with a strip of mucous membrane from the whole of the greater curvature of the stomach embedded in a paraffin block $1\frac{1}{2}$ inches square, while the individual sections were accommodated on a 3 by 2 inch glass slide.

The strip of tissue is loosely wound round a cylindrical spigot of wood 2 inches long and $\frac{1}{4}$ inch diameter to form a coil. In a single strip there is no need to be concerned with the mode of coiling the tissue, but where a number of strips are taken in seriation, e.g., the whole intestine, it is desirable to maintain a fixed convention of starting the coil in such a manner that the inside of the coil is always the proximal end of the segment of tissue.

The coiled up specimen now resembles a watch spring. A pin is thrust through the tissue opposite the cutting surface to prevent it from unrolling. The pin originally was made to pierce the wood to provide a better anchorage, but this procedure was subsequently dispensed with, as any additional manipulation is bound to prolong the postmortem time. Avoidance of any delay in fixation is particularly important in the bowel where Macklin and Macklin (1926) have shown the agonal and postmortem changes supervene with great rapidity.

The tissue may be fixed in any of the routine fixatives, such as formol or Zenker-formol, after which the wooden spigot is removed.

Occasionally one finds after Zenker-formol fixation that the central portion of tissue is incompletely fixed owing to faulty penetration by the fixative. To prevent erratic fixation the whole viscus may be removed and fixed without preliminary rolling, and later wound as described above and returned to the fixative in order to preserve the coiled up character of the tissue. This procedure is less satisfactory than fixation from the outset of a strip of tissue in its coiled state.

HEMOPHILIA, Investigations in, Bendien, W M, and van Creveld, S. *Am J Dis Child.* 54: 713, 1937

The fact that in normal fresh plasma and serum a substance is present which exerts a coagulation promoting influence on hemophilic plasma and blood is discussed.

The question of for which protein fraction of the serum or the plasma this substance shows the greatest affinity is discussed. Though the substance must be present in the coarse disperse protein fraction, it undoubtedly is not euglobulin itself.

A method is described for precipitation of the coagulation promoting substance from normal fresh serum by slight acidifying.

A simpler method is also described, i.e., adsorption and elution, by which the coagulation promoting substance can be obtained from fresh normal serum in a medium poor in proteins.

By dissolving the coagulation globulin (which was precipitated from the serum by slight acidifying) in water or in physiologic solution of sodium chloride to which has been added 3 or 4 per cent of sodium carbonate, it can be dissolved into a volume which is ten times as small as the volume of the normal serum from which it has been prepared. This solution is free from cholesterol and lipid phosphorus.

The solutions as obtained by the method described showed an activity which was about five times as great as that of the fresh serum itself. Moreover, these solutions remained active much longer than the serum itself when kept in the refrigerator.

The results obtained with oral, intramuscular, and intravenous administration of solutions of the coagulation promoting substance to three patients with hemophilia are described. Especially by application of the intravenous method distinct results were obtained. In one patient we succeeded repeatedly in keeping the coagulation time within normal limits for some days by an intravenous injection.

SICKLE CELL ANEMIA, Immunologic Studies of, Cardozo, W. W. *Arch Int Med.* 60: 623, 1937.

A total of 1,570 patients were tested for sickling of red blood cells. The group consisted of both adults and children. There were 1,263 negroes (94.2 per cent who showed sickling) and 307 non negroes (0.32 per cent).

The blood type of 1,560 patients tested for sickling was determined by means of the standard laboratory "slide method." The percentages of each blood group in the series of patients who showed sickling was compared with similar groups among the normal negroes and non negroes. In general there was no marked tendency for the patients who showed sickling to be concentrated in any one blood group.

The distribution of the immune agglutinogens M and N was determined for 306 non negroes, 209 normal negroes and 63 patients who showed sickling. The distribution of these immune agglutinogens in the patients who showed sickling corresponded, within limits, to that of the normal persons of both races.

An attempt was made to detect a specific immune agglutinin for those who showed sickling. By the method used no specific agglutinin was found.

The anomalous isohemagglutinins which were found by another investigator in patients who showed sickling were not found in this series by cross matching the cells and serum of patients who were known to show sickling against the cells and serum of the normal persons of both races.

Tests confirmed the work of Huck and Hahn, in which they found that serum is unnecessary in producing the sickling phenomena.

It is shown further in the same tests that the sickling factor remains within the cell, no matter how long preserved, as long as the cell itself remains intact.

Individual analysis of the entire series of 120 patients who showed sickling was made, and reports of 2 cases are given.

BLOOD, Convenient Method for Securing for Analysis, Abrahamson, A. *Science* 86: 202, 1937.

After pricking the finger with a lancet, allow the blood to drop onto a petrolatum block with a depression on it. Slight pressure proximal to the wound or a rubber band placed round the finger will produce free flow, and as much as 1 c.c. of blood may be obtained readily. The blood can then be drawn up into the pipette. The blood will not clot on the petrolatum in the short time necessary for collection. A muffin tin, consisting of six depressions in a tin plate, is used in making the petrolatum blocks. The depressions are filled with melted petrolatum and then set aside to cool. The contraction of the paraffin in cooling will produce a smooth depressed surface that serves admirably for the collection of the blood.

BILIRUBIN, Simple Reaction for Demonstration of, in Urine, Von Purjesz, B. *Med. Klin.* 33: 1271, 1937.

From 2 to 3 c.c. of urine is placed in a test tube and is mixed with 2 c.c. of a 20 per cent solution of sulfosalicylic acid. Then from 2 to 5 drops of a 30 per cent solution of hydrogen peroxide is added. After shaking, the mixture is left standing; the green coloration appears after a few minutes or, at the latest, after fifteen minutes. The intensity of the green depends on the quantity of the bilirubin; if the bilirubin content is slight, there appears an olive green instead of a grassy green. If the urine is free from bilirubin, a reddish color develops. The presence of considerable quantities of blood impairs the reactions, but this shortcoming can be overcome by filtration.

ANEMIA of Chronic Glomerulonephritis and Its Relationship to Gastric Acidity, Townsend, S. R., Massie, E., and Lyons, R. H. *Am. J. M. Sc.* 194: 636, 1937.

The anemia of chronic glomerulonephritis is normocytic in type.

The anemia becomes manifest as renal insufficiency occurs and increases with the degree of nitrogen retention.

With increasing anemia, there is a decrease in the gastric acidity.

Absolute achlorhydria is present when the carbon dioxide content of whole blood (plasma bicarbonate) falls below 30 volumes per cent.

There is no apparent lack of active blood forming tissue.

A discussion of the relationship between renal insufficiency, gastric acidity, and the anemia has been given.

Reasons have been offered for the persistence of the anemia and its refractoriness to therapy.

POISONING, Fatal Nicotine, Beeman, J. A., and Hunter, W. C. *Arch. Path.* 24: 481, 1937.

Twenty-four fatal cases of nicotine poisoning due to drinking insecticides are presented. Black Leaf 40, a commercial insecticide containing nicotine, produces hemorrhagic gastritis. Unusual hyperemia of the kidneys, or the presence of a brownish froth about the mouth and nose should suggest nicotine poisoning. Such poisoning, even when the drug has been taken in large amounts, is not necessarily associated with gastritis or with an odor of nicotine. The local irritant action of Black Leaf 40 is not due to its nicotine content.

LEUKEMIA, Classification and Terminology of, and Allied Disorders, Forkner, C. E. *Arch. Int. Med.* 60: 582, 1937.

The confusion existing in the classification and terminology of diseases of the blood-forming organs, particularly of leucemia and related disorders, is reviewed and discussed.

A classification is presented which encompasses all known variants of leucemia and which simplifies the concepts of these disorders.

CLASSIFICATION OF LEUCEMIA (LEUCEMIA, LEUCOSIS, LEUCOCYTHAEMIA)

CLINICAL DESIGNATION	GENERAL TYPE OF LEUCEMIA	CELL OF ORIGIN	SPECIFIC TYPE OF LEUCEMIA	SYMPTOMS DEPENDING ON COMMON USAGE, ON COURSE OF DISEASE, OR ON CLINICAL OR HEMATOLOGIC CHARACTERISTICS	
				NEUTROPHILOCYTIC LEUCEMIA	Myelogenous, myeloid, myelocytic or myeloblastic leucemia; myelosis
Leucemia or subleucemic (aleucemic) leucemia (acute or chronic)	Myelogenous (arising from cells or bone marrow)	Myeloblast	Eosinophilic leucemia	Eosinophilic leucemia	Eosinophilic leucemia
			Basophilic leucemia	Basophilic leucemia	Basophilic leucemia
			Chloroleucemia	Chloroleucemia	Chloroma or chloroleuco-sarcoma
			Erythro-leucemia	Erythro-leucemia	Leucemia associated with erythremia
Lymphogenous (arising from cells of lymphoid tissue)	Lymphoblast	Lymphoblast	Megakaryocytic leucemia	Megakaryocytic leucemia	Lymphogenous lymphoid, lymphatic or lymphoblastic leucemia; lymphoblastoma leucemicum, lymphadenosis
			LYMPHO CYTIC LEUCEMIA	LYMPHO CYTIC LEUCEMIA	Lymphogenous lymphoid, lymphatic or lymphoblastic leucemia; lymphoblastoma leucemicum, lymphadenosis
			Leuco-sarcoma	Leuco-sarcoma	Lympho-sarcoma associated with anemia
Lymphogenous or myelogenous	Primitive mesenchyme cell	Primitive mesenchyme cell	Stem cell leucemia	Stem cell leucemia	Hemohistioblastic, embryonal or lymphoidocytic leucemia
			Plasma cell myeloblast and lymphoblast	Plasma cell leucemia	Plasmacytoma with leucemia or multiple myeloma with leucemia
Disputed	Monoblast	Monoblast	MONOCYTIC LEUCEMIA	MONOCYTIC LEUCEMIA	Histiocytic leucemia; reticulosis; reticulo endotheliosis; reticulum cell leucemia; reticulosarcoma

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Narrative of an Investigation Concerning an Ancient Medicinal Remedy and Its Modern Utilities*

THE author presents his experiences and results with the use of Comfrey root as a therapeutic agent. Having employed an infusion of the root to several cases of chronic indolent ulcers, and having obtained satisfactory results, he directed his attention to an analytical determination of the root's constituents. He found that allantoin was the active principle and could be recovered from the root in crystalline form. Allantoin is a substance present in the fetal environment and in the roots of plants. Its concentration in these two places rapidly diminishes toward the end of pregnancy and toward the end of the growth period of the plant. This would lead one to suspect that allantoin is utilized by plants and animals during their period of physical development. The author presents cases in which favorable results followed the application of allantoin, both internally and externally, to degenerative lesions. Injection of allantoin into the bulbs of plants hastened their growth and caused an increase in the size of their flowers. Evidence is presented which indicates that allantoin stimulates leucocyte formation. In many cases of lobar and bronchopneumonia, in which allantoin was used therapeutically, the crisis seemed to be hastened. There are chapters devoted to the chemistry of allantoin and to the methods used in its preparation and administration. The author points out that maggots have been widely used in the past for the purpose of clearing up dead tissue, and that the excreta of maggots contain a high concentration of allantoin.

A Textbook of Histology†

WHEN this book was prepared, it was intended to be used as a textbook for college students. The attention of the college student interested in biology has been, for the most part, directed toward a study of general biology, comparative anatomy, embryology, and genetics. Little or no attention has been paid to the subject of histology. As the author points out, the broader and more general biological concepts may be much more satisfactorily presented to a college student through the medium of the study of the cells themselves, since a sound knowledge of cellular makeup is essential for the fullest understanding of all biological processes. In this book the material is lucidly presented, and the contents are easily and smoothly handled throughout. The author does not exhaust his reader with detailed reports of controversial minutiae, which are of interest only to those devoting their time to research. The book is replete with photographs and drawings of tissue sections. Although this work is intended primarily as a textbook for college students, it will be a valuable reference book for any physician who wishes to refresh his memory concerning the number of important details of the structure of the human body.

*Narrative of an Investigation Concerning an Ancient Medicinal Utilities. The Symphytum Officinale and Its Contained Allantoin. By M.D., F.R.C.P., Hon. Consulting Physician to the Liverpool Royal St. Royal Liverpool Children's Hospital and to the Moore Cottage Hospital. Water. Together with an Account of the Chemical Constitution of Allantoin. By A. W. Titherley, D.Sc., Ph.D., formerly Lecturer on Organic Chemistry in the University of Liverpool. Paper, 60 pages. John Bale, Sons & Danielsson, Ltd., London, 1936.

†A Textbook of Histology. By Joseph Krafka, Jr., Ph.D., M.D., Professor of Microscopic Anatomy, University of Georgia, School of Medicine, Augusta. Cloth, 246 pages. \$2.50. Williams and Wilkins Co., Baltimore, Md., 1936.

Amino Acid and Ammonia Metabolism in Liver Diseases*

THE purpose of this monograph is to investigate protein metabolism in liver disease. As the author points out, the vast majority of previous experimental investigation has been unreliable due to the use of faulty analytical technique. As a result, there has been a clouding rather than a clarification of our knowledge concerning the changes in protein metabolism in conditions associated with an abnormally functioning liver. The author sets forth in detail his methods of procedure and technique. He is concerned with amino acid metabolism and ammonia metabolism in normal and abnormal conditions. He reviews previous investigation, and sets forth the results of his own efforts. There is a presentation of case histories of acute hepatitis, liver cirrhosis, obstructive jaundice, and other similar conditions, all correlated with a discussion of the clinical significance of the ammonia tolerance test as a diagnostic aid in their detection. The entire work is clearly and scientifically presented. It will surely be of great assistance for the understanding of liver function and the diagnosis of pathologic conditions of the liver.

Diathermy†

THIS new edition has been revised and expanded by the inclusion of accounts of short wave and inductothermic treatment.

The scope of the volume is indicated by the section headings.

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Part III Surgical Electrothermic Methods and Their Uses

To those wishing an authoritative survey this book can be recommended as comprehensive and intelligible.

Practical Methods in Biochemistry‡

THIS is a practical manual for students to whom it may be recommended.

The Compleat Pediatrician§

IN A PREVIOUS review of the first edition of this book it was commended as an exceedingly practical and valuable contribution to pediatric literature. This second edition is, in many respects, even a more useful volume than its predecessor not only because it is enriched by the addition of data culled from the pediatric literature of the past four years, but also because the book has been reorganized to make its contents even more readily accessible.

As before, the plan of the book is reminiscent of a thesaurus. In this edition, however, symptoms and diseases have been divided into seven chapters on the basis of the anatomic system chiefly involved, and the number of paragraphs has been reduced.

The book is not, of course, an automatic diagnostic machine but serves as a reminder of various diagnostic possibilities.

*Amino Acid and Ammonia Metabolism in Liver Diseases. By Esben Kirk. Paper 147 pages. Levin & Munksgaard, Copenhagen, 1936.

†Diathermy, Including Diathermotherapy and Other Forms of Medical and Surgical Electrothermic Treatment. By Elkin P. Cumberbatch, Medical Officer in Charge of Electrical Department and Lecturer on Medical Electricity, St. Bartholomew's Hospital, with nine collaborators. Cloth ed. 3, 576 pages, 168 figures. \$6.00. William Wood & Co., Baltimore, Md.

‡Practical Methods in Biochemistry. By Frederick C. Koch, Professor of Biochemistry, University of Chicago. Cloth ed. 2, revised, 301 pages. William Wood & Co., Baltimore, Md.

§The Compleat Pediatrician: Practical Diagnostic Therapeutic and Preventive Pediatrics. By W. C. Davison, Professor of Pediatrics, Duke University School of Medicine. ed. 2. Completely rewritten. Cloth \$3.75. Duke University Press.

As before, this volume can be recommended as based upon comprehensive and digested experience and as perhaps the most comprehensive index to pediatric practice yet made available in a single volume.

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Diseases of the Skin*

THIS is an excellent book which can be recommended without reserve. The illustrations and colored plates maintain a high order of excellence in keeping with the character of the text.

The practitioner will make no mistake in adding this volume to his working book-shelf.

Demonstration of Physical Signs in Clinical Surgery†

IF THERE is any better book of this kind it has not been the good fortune of this reviewer to encounter it.

It is only necessary to say that this new edition is of equal, if not greater, excellence than its predecessors.

Treatment of Some Chronic and Incurable Diseases‡

THE purpose of this book is to discuss measures applicable to those conditions which, because they are classified as "chronic or incurable," are often left untreated by the physician and so left to the tender mercies of various cults and the like.

It is Dr. Todd's purpose to emphasize that even in chronic or incurable diseases it is often possible, through symptomatic treatment, to add greatly to the patient's comfort. His viewpoint and experience are entirely British and thus, in not a few ways, at variance with the methods and experience of American medicine. His views on the management of diabetes, for example, are hardly in accord with those generally held. He may be classed as a poly-therapist for he employs rather complex mixtures and drug combinations. Thyroid extract, colloidal sulfur, fecal vaccines, and a variety of patented foods and proprietary preparations, many of them unknown in America, are frequently encountered.

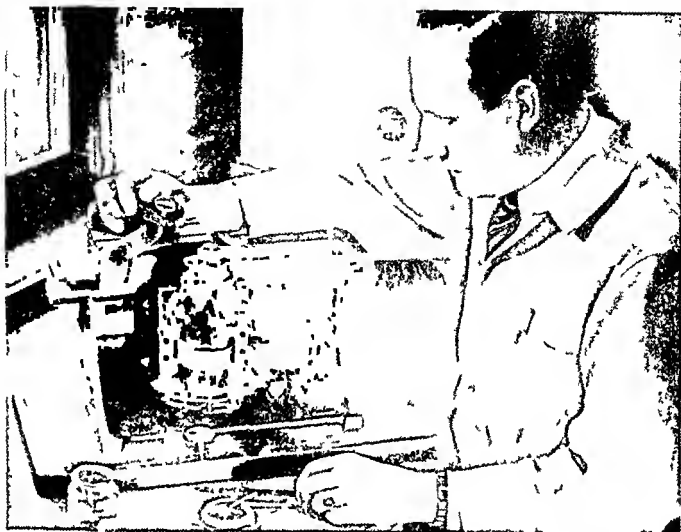
Probably the best chapter in the book is that on the management of constipation.

Principally because of its rather insular viewpoint it is doubtful whether this book will appeal greatly to the American physician.

*Diseases of the Skin. A Manual for Students and Practitioners. By the late Robert V. MacKenna, M.A., M.D., Ch. B. (Edin.), ed. 4. Revised and enlarged, by Robert M. B. MacKenna, M.A., M.D. (Camb.) M.R.C.P. (Lond.) Honorary Dermatologist, Royal Southern Hospital, Liverpool; Honorary Dermatologist, Liverpool Stanley Hospital. Cloth, 537 pages, 163 figures, 46 colored plates, \$7.00. William Wood & Co., Baltimore, Md.

†Demonstration of Physical Signs in Clinical Surgery. By Hamilton Bailey, F.R.C.S. (Eng.), Surgeon, Royal Northern Hospital, London; Surgeon and Urologist, Essex County Council; Surgeon, Italian Hospital; Consulting Surgeon, Clacton Hospital; External Examiner in Surgery; University of Bristol, etc. Cloth, ed. 6, 258 illustrations, \$6.50. William Wood & Co., Baltimore, Md.

‡Treatment of Some Chronic and Incurable Diseases. By A. H. Todd, M.D., Honorary Physician, Bristol Royal Infirmary. Cloth, 203 pages, \$3.00, William Wood & Co., Baltimore, Md.



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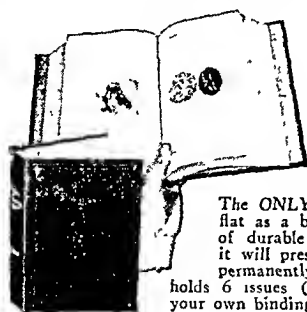
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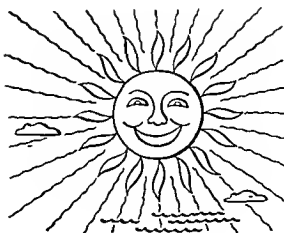
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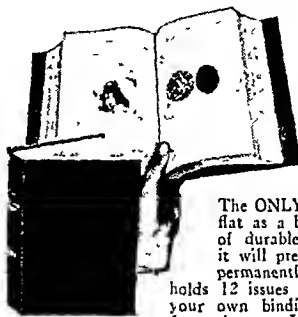
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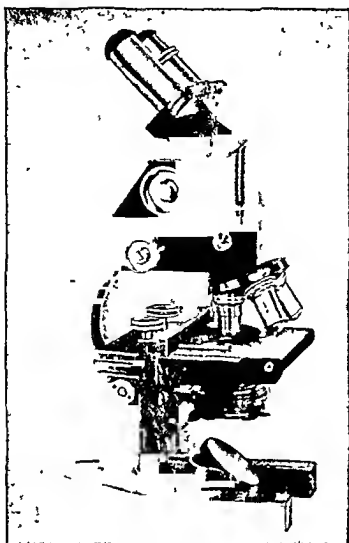
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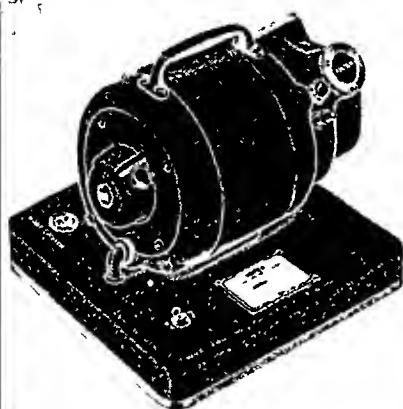
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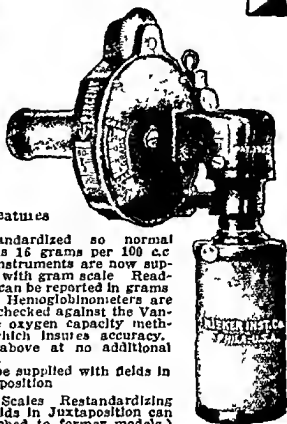
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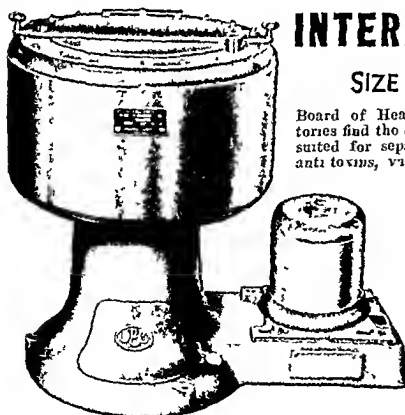
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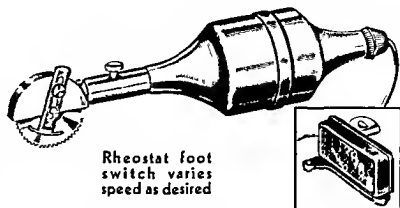
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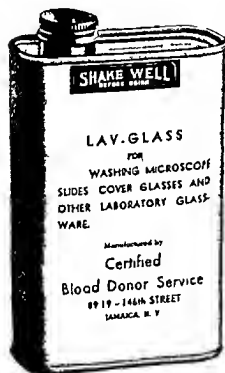
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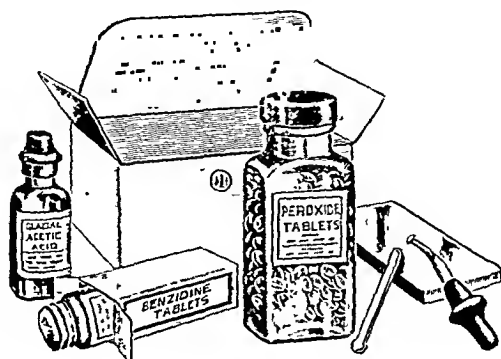
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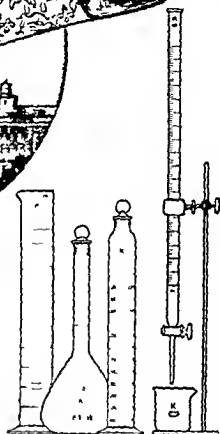
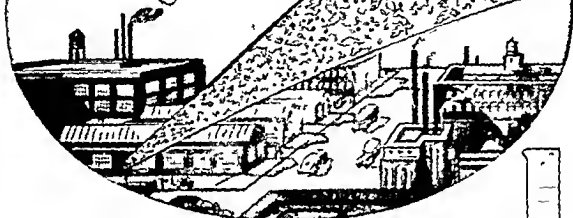
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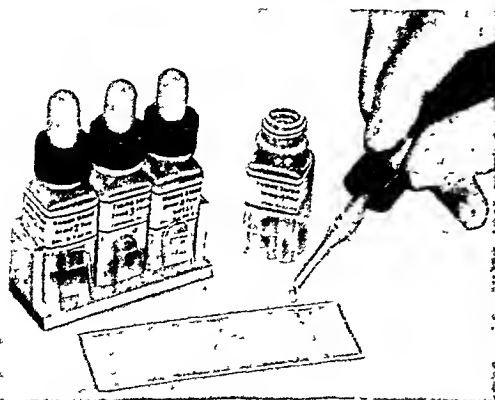
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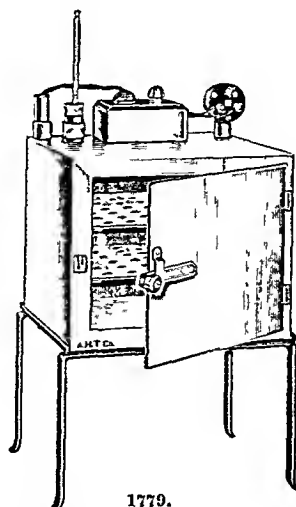
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INTRAVENOUS AUTOHEMOTHERAPY WITH HEMOLYZED BLOOD TECHNIQUE AND LEUCOPOIETIC RESPONSE

PRELIMINARY REPORT*

STANLEY ROCHELLE DEAN, M D, AND H C SOLOMON, M D, BOSTON, MASS

INTRODUCTION

THE present report, while complete in itself, is intended to serve as an introduction to a more intensive study concerning the mechanism of malaria in ocular neurosyphilis, the principal text of which will be reported separately.

The modern vogue of treating neurosyphilis with mechanical hyperpyrexia has focused the attention of investigators, with renewed emphasis, upon the question of whether the beneficial results in these cases are dependent upon fever per se or upon fever plus a biologic factor such as merozoites in malaria. Any study of the mechanism of malaria must take into account the fundamental constituents which go to make it up. Of these there are at least three, each of which is apparently capable of provoking a defensive reaction in the organism. These are (1) the malarial parasite, (2) fever, and (3) blood destruction.

One of these—fever—we can already duplicate at will. We reasoned that if we could introduce another constituent, such as the factor of blood destruction we might approach a step nearer to the artificial duplication of malaria. It seemed logical to attempt this by giving a group of patients intravenous injections of hemolyzed autogenous blood just prior to the induction of fever al-

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though, as far as we know, no one before has attempted to break down the problem in just this manner. Various pertinent observations were then planned which would lend themselves to comparison with certain results known to be typical of malaria. Our first investigation was directed toward the alterations in the cellular equilibria of the organism. For this purpose each patient was observed during four separate periods, of which this report concerns itself only with the first two: (1) A control or basal period, (2) a period of autohemotherapy alone, (3) a period of pyretotherapy alone, and (4) a period of autohemopyretotherapy; this is a term coined by us to conveniently designate the combination of autohemotherapy and induced fever. We hope, incidentally, that those investigators who are familiar with the excellent results sometimes obtained with autohemotherapy in various morbid processes, will recognize in combined autohemopyretotherapy a new therapeutic and experimental approach to the problems of enhancing the defensive mechanism of the organism. Although a considerable literature has accumulated about autohemotherapy, surprisingly little has been written about its intravenous use, especially with hemolyzed blood.

Desearpentries^{1, 2} employs distilled water and blood in a proportion of 2:1, lysing 10 c.e. uneitrated blood in 20 c.e. distilled water; he then agitates the mixture in a flask containing several marbles, about which the clumps of fibrin adhere; finally, he injects the clear fluid subcutaneously in divided doses—a momentarily painful, but, according to him, a very beneficial procedure in various septic conditions. He believes that, among other effects, the hemopoietic system is stimulated, but offers no data in support of this view.

Zimmerman³ cautiously adds 2 c.e. distilled water to 20 c.e. blood and immediately reinjects it either intravenously or intramuscularly. He states that there is *usually* a decrease in the total number of leucocytes, eosinophiles, and lymphocytes; but that the neutrophils exhibit a relative increase. Frey⁴ believes that lysis, following the intravenous injection of hemolyzed blood, is prolonged in the blood stream, but Zimmerman does not agree with that view.

Brunner and Breuer⁵ place 14 c.e. distilled water into a 20 c.e. syringe, aspirate blood into the same syringe to its capacity, then, without drawing the needle out of the vein, reinject the mixture.

Thus it may be seen that there is considerable divergence of opinion regarding technique and physiologic results; one is led to believe that the choice of technique has been empirical rather than based upon an experimental foundation. In fact, we were able to obtain only very meager information in answer to the three fundamental questions that we had asked ourselves, viz.:

1. What is the average minimal hemolytic ratio of distilled water to blood?
2. How much hemolyzed autogenous blood can be injected intravenously, allowing a fair margin of safety?

3. What is the nature of the leucopoietic response, if any, to such injections?

We shall, therefore, endeavor to answer these and other concomitant questions as a result of our own investigations. In so doing, this report hopes to establish a rational basis for the technique of intravenous autohemotherapy, either experimental or therapeutic.

PROCEDURE

I *Determination of Minimal Hemolytic Ratio*—Graduated amounts of distilled water, ranging from 0.2 cc to 5 cc were placed in a series of test tubes. To each tube, 2 cc of venous blood were then added, using nonclotted blood in one series, and clotted blood in another series. The progress of hemolysis was then observed in each tube, both macroscopically and microscopically.

In the nonclotted series it was observed that hemolysis began when the proportion of water and blood was 1:1, but proceeded slowly and incompletely due to the fact that numbers of red blood cells became enmeshed in the fibrin clumps, where they were not so well exposed to the action of the distilled water.

In the clotted series a more favorable course of events took place. Here also the minimal hemolytic ratio was 1:1, but, there being no fibrin clumps, all the erythrocytes were exposed to the distilled water, with the result that hemolysis was much more rapid and complete.

Having determined that the minimal hemolytic ratio was approximately 1:1, we tried adding a little whole blood to a mixture of that proportion in order to see if any further hemolysis would occur, however, the added blood merely settled to the bottom of the tube without hemolyzing.

A practical point to keep in mind is this. When blood is mixed with the proper proportion of distilled water, hemolysis occurs very rapidly, and the mixture becomes fluid and homogeneous almost at once, but if allowed to stand for any length of time at room temperature it begins to stiffen and jelly slightly. Therefore, the optimum time to reject the mixture is within ten minutes after mixing.

II *Determination of Amount of Injection*—We know from Ross's estimation of the number of parasites present during a malarial paroxysm, that a considerable amount of blood must be destroyed during each chill. It seemed to us therefore, that much larger amounts of lysed blood than indicated in the literature could be injected with impunity. Working first with rabbits, we found that they could easily tolerate an injection of as much as 52 cc of total solution, consisting of 20 cc blood, 2 cc citrate, and 30 cc distilled water.

Thus encouraged, we then proceeded with similar experiments in human beings. As stated above, the average minimal hemolytic ratio between blood and distilled water was found to be 1:1. In order to be perfectly accurate, one should, no doubt, determine the hemolytic ratio in each patient treated, however, in this type of experiment average values are adequate. We therefore decided to employ routinely a proportion of three parts distilled water to two parts blood, feeling that such a mixture would cause optimal hemolysis without too great an excess of distilled water in the majority of cases.

In all, seven patients were observed, their ages varied from 30 to 57 years. The diagnosis in each case was general paresis. Some had received fever therapy prior to the present investigation. These and other data are summarized in Table I since they may have a possible bearing upon the results obtained. Certain clinical data, such as temperature, pulse, blood pressure, and subjective sensations, were also recorded for several hours before and after the injections.

TABLE I

NAME AND GROUP	DIAGNOSIS	AGE	DURATION OF SYPHILIS	HOURS OF FEVER 103° TO 106°	AMOUNT OF INJECTION IN C.C.			
					BLOOD	CITRATE	DIS- TILLED WATER	TOTAL
H.F. (A)	General paresis	36	?	46	10	1	15	26
G.F. (A)	General paresis	32	12 yr.	Malaria	20	2	30	52
C.K. (B)	General paresis, optic atrophy, retinitis	57	(?) 18 yr.	46	30	3	45	78
C.C. (A)	General paresis	38	13 yr.	80	30	3	45	78
R.B. (A)	General paresis, cachexia	37	?	0	40	4	60	104
W.S. (B)	General paresis, 6th nerve palsy	50	(?) 18 yr.	28	50	5	75	130
S.E. (B)	General paresis, funnel chest, tachycardia	30	(?) 12 yr.	0	50	5	75	130
Average		40	(?) 15 yr.	33	33	3.3	50	86

The amounts of hemolyzed blood injected ranged from 10 c.c. to 50 c.c., the average being 33 c.c. The technique is quite simple. Into a 100 c.c. syringe is placed enough sodium citrate solution to make up 10 per cent of the amount of blood that is withdrawn from the vein. This citrated blood is left in the syringe, and, after sticking a sterile cork over the point of the needle to prevent leakage, is shaken vigorously for about five minutes. Then one and a half times as much distilled water is drawn into the same syringe to make a proportion of 3:2, and the mixture is again agitated for five minutes. Finally the entire solution is reinjected intravenously. If the total amount of the solution is more than the syringe can hold, the distilled water may be placed in a beaker and the blood mixed with it by swishing it back and forth through the syringe. Aside from warming the distilled water to body temperature and keeping the entire procedure aseptic, no other special precautions are necessary.

RESULTS

Not the slightest discomfort was experienced by any of the patients following the injection. In no case was there even the semblance of a chill or a sign of jaundice. The temperature and pulse rate were not affected. Blood pressures in a few cases showed transient changes, but these were so slight and inconstant that no significance has been attached to them.

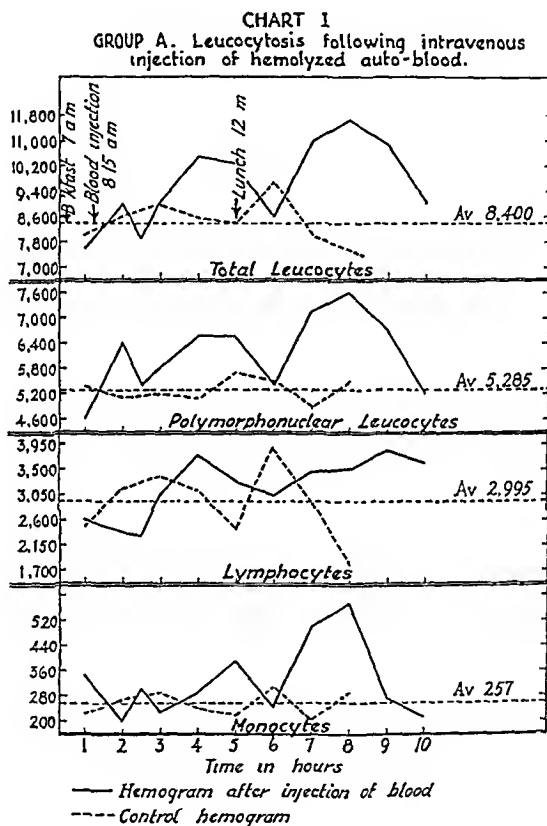
III. The Leucopoietic Response. A. Method of Determination.—Before attempting to evaluate the leucocytic response to any pathologic or chemical disturbance, it is important first to determine an adequate normal base line derived from frequent successive readings, for it is now recognized that continual fluctuations occur in the white blood cells even under physiologic conditions. Accordingly the following method of comparison was adopted. Each patient was observed during an eight- or ten-hour period, usually between 8:00 A.M. and 3:00 to 5:00 P.M., on two successive days; the first day served as a control period, the second day a period of autohemotherapy. All other factors were kept as constant as possible. The patient was kept in bed and allowed a regular house

TABLE II

GROUP	PATIENT	TOTAL LEUCOCYTES			POLY MORPH CELLS			LYMPHOCYTES			MONOCYTES		
		CONTROL	BLOOD INJECTION	PER CENT CHANGE	CONTROL	BLOOD INJECTION	PER CENT CHANGE	CONTROL	BLOOD INJECTION	PER CENT CHANGE	CONTROL	BLOOD INJECTION	PER CENT CHANGE
A (Leuco cytosis)	H B	8,800	10,500	19	5,000	6,900	23	3,200	3,700	10	80	316	267
	G F	9,000	10,300	14	4,800	5,800	21	3,800	4,100	10	379	379	NS
	C O	8,400	9,500	13	5,700	6,500	14	2,500	2,600	NS	234	248	NS
	H. F.	7,900	8,700	10	4,900	6,000	23	2,600	2,600	NS	340	404	19
Average for Group A		8,500	9,800	14	5,200	6,300	21	3,000	3,200	NS	260	337	30
B (Leuco penia)	C K	8,800	7,100	19	5,400	3,900	28	3,100	2,900	NS	302	270	NS
	W S	8,400	5,200	28	5,500	3,100	42	2,800	2,100	95	95	35	63
	S E	9,300	7,500	17	5,400	4,300	20	3,700	3,500	NS	189	155	65
	Average for Group B	8,800	6,700	23	5,400	3,800	20	3,200	2,800	13	195	135	40

diet. Blood counts and films were obtained at thirty- to sixty-minute intervals during each eight- to ten-hour period, using Bureau of Standards pipettes and counting chambers.

Altogether a total of 115 blood specimens were taken; 51 during the control periods and 64 during the injection periods. With the above facts in mind, we feel that the averages of our numerous readings afford a reasonably accurate criterion for estimating our results, and that any alteration of the basal average amounting to 10 per cent or more can be looked upon as the specific result of the autogenous blood injections.



B. Results.—We found that our patients fell into two groups: Group A (four patients) exhibited an *increase* in all the leucocytic elements following autohemotherapy. Group B (three patients) showed a *decrease*. The averages and percentage changes for each patient are summarized in Table II. Only changes of 10 per cent or more are recorded numerically, those below 10 per cent being marked N. S. meaning “not significant.”

Taking up each element of the hemogram separately, first in Group A, it will be noticed that the total white cell count displays elevations, varying from 10 to 19 per cent; of the differential elements, the polymorphonuclear cells reveal the most consistent increases, varying from 14 to 23 per cent; the lymphocytes exhibit significant rises in two cases; while the monocytes develop the

Group A

1. *Total Leucocytes*.—Within the first hour after injection, the white count develops a slight increase of 7 per cent above the basal average, then drops during the second hour to nearly the same level below the base line. Another peak is reached during the fourth hour with an increase of 25 per cent above normal; this is again followed by a depression. Then there is another gradual ascent until at the eighth hour the highest peak is reached, 40 per cent above the base line. Ten hours after the injection the count has fallen considerably, but still remains a little above the basal level.

2. *Polymorphonuclear Leucocytes*.—The pattern of this curve follows that of the above very closely, although the quantitative changes are somewhat greater. As in the first curve, the highest peak is reached in the eighth hour, this time rocketing 44 per cent above the basal level. The qualitative resemblance in the patterns of these two curves is in itself a hint that the changes in the hemogram are due primarily to the lability of the polymorphonuclear cells more than any other leucocytic constituent. This fact is corroborated by the numerical data in Table II.

3. *Lymphocytes*.—Here the pattern of the graph changes considerably. During the first two hours there is a progressive decrease to 30 per cent below the basal average. Not until the third hour does the count begin to rise, but then it ascends until at the fourth hour it has reached a peak of 23 per cent. This is followed by a gradual depression, then another elevation until it reaches a second peak, 27 per cent above the base line in the ninth hour. Thus, the lymphocyte curve lags at least an hour behind the polymorphonuclear cell curve at every point, again demonstrating the more sensitive lability of the latter type of cells.

4. *Monocytes*.—The fluctuations in these cells are much more irregular and inconstant than the others, and are therefore more difficult to describe. Just as in the case of the other cells, the highest peak occurs late, in this case ascending to 120 per cent above the normal average in the eighth hour.

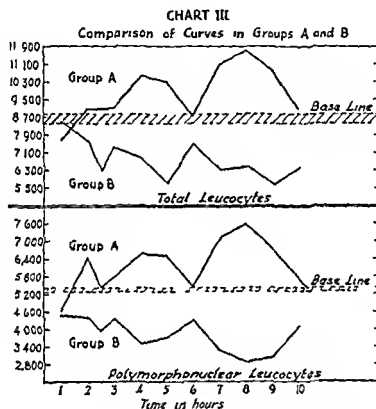
Group B

1. *Total Leucocytes*.—Group B differs from Group A not only in direction but also in intensity; i.e., it develops leucopenia following autohemotherapy, and this leucopenia is quantitatively greater than the leucocytosis of Group A (see Table II).

The initial reaction following injection is a progressive diminution in the white cell count until a point is reached 30 per cent below the base line during the second hour. Thereafter, just as in Group A, there are a series of peaks and depressions, never, however, rising above the base line, and finally reaching a low point of 36 per cent during the ninth hour. In fact, the reaction is *diametrically opposite to that of Group A*. Although at first glance nothing could seem more remote, closer examination reveals the peculiar fact that the patterns of this and the polymorphonuclear cell curves are almost mirror images of the same respective curves in Group A (see Chart III).

2 *Polymorphonuclear Leucocytes*—Again the initial reaction is a drop, followed by the usual succession of peaks and valleys, the latter descending to lower levels each time and finally reaching the lowest point, 46 per cent below the basal level in the ninth hour. Above has been mentioned the curious fact that this curve is almost the direct reverse of the polymorphonuclear cell curve in Group A. If the reader will superimpose Chart I upon Chart II in such a manner that the base lines of the "poly" curves coincide and the corresponding peaks and depressions point in the same direction, the amazing similarity in the patterns of the two curves will be strikingly demonstrated (see also Chart III).

3 *Lymphocytes*—Here also the initial reaction is a drop in the second hour, followed by a series of rises and falls. The greatest drop, in this case 47 per cent, occurs in the fifth hour.



4 *Monocytes*—Contrary to the three previous graphs, the initial reaction here is a sharp ascent to a peak 80 per cent above the basal level at the end of the first hour. Here also we see the characteristic peaks and depressions, the lowest point being reached in the sixth hour.

The tenacity of the leucopenia throughout Group B is evidenced by the fact that none of the curves reach the control base line even ten hours after the injection.

DISCUSSION

In our hands the intravenous injection of relatively large amounts of hemolyzed autoblood has been singularly free of discomfort, pain, or any other apparent ill effects. Even the feeble patients, R. B. and S. E., whom we regarded with considerable trepidation, fared no worse than their hardier brethren. Although we did not exceed 50 cc of blood or a total of 130 cc of hemolyzed solution in this series, we have reason to believe that larger amounts may be used without harm. Attention is called to the safety of the procedure and the

simplicity of technique not only for its therapeutic but also for its experimental implications; thus, the clinician and the hematologist will recognize in this medium an interesting means of studying such morbid conditions as hemolytic icterus, blood-destroying diseases like malaria, the mechanism of leucopenia and leucocytosis, etc.

A few facts already touched upon may require further comment in our discussion. There is, for example, the question of the use of citrated versus whole blood. Desearpentries^{1, 2} emphatically denounces the use of anticoagulants, claiming that any substance that disturbs the physiologic functions of the blood to the point of preventing coagulation, must equally disturb other functions whose aim it is to defend the organism against infection. It would seem, however, that his objection is more hypothetical than real. The lysis of blood by distilled water in itself produces a profound alteration in physiologic functions, and it is difficult to conceive how the addition of a relatively innocuous anticoagulant can have any more deleterious effect. Besides, there is an important advantage in using an anticoagulant, since the process of hemolysis is hastened thereby, and the technique greatly simplified.

Another subject for discussion deals with the question of prolongation of hemolysis in the blood stream. Our experiments have indicated that, at least *in vitro*, when more blood is added to a hemolytic system in equilibrium, further hemolysis does not occur. When, as in our procedures, we employ a small excess of distilled water, i.e., a ratio of 3:2, it is possible that very slight additional hemolysis occurs at the moment of injection, but there is no evidence to indicate that it is prolonged, and for practical purposes it is so insignificant that it may be ignored.

So much for the details of technique. Thus far we have been dealing with self-evident facts that require little elaboration. We come now to the second phase of our work, namely, the leucopoietic response—a phase that is easier to describe than to interpret. In fact, any attempt to arrive at a final interpretation at this time could only result in a pedantic flight into the realms of abstract speculation far beyond our immediate horizon. Therefore, until we have collected more cases, we will confine ourselves in this report to a simple presentation of facts, at the same time raising such questions and making such suggestions as may seem pertinent to the material at hand.

The first and most important question that demands consideration is this: Why does one group of individuals develop leucocytosis and another leucopenia following the intravenous injection of hemolyzed autoblood? In Table I are listed a few of the factors that might conceivably influence the type of reaction; but careful examination of these factors throws little light upon the problem. Thus, the presence of complicating disease, the age of the patient, and the duration of syphilis show a similar distribution throughout both groups. We wondered if repeated bouts of fever might lessen the hematopoietic response. We found, however, that patients R. B. and S. E., although receiving no fever at all, fell into two different groups; and that C. C., in spite of eighty hours of fever to his credit, developed a leucocytosis.

Does the amount of blood that is injected have any influence upon the type of reaction? W S and S U, who received the largest amounts of blood, both developed leucopenia, but, on the other hand, C K, with only 30 cc of blood also developed leucopenia, whereas R B with 40 cc of blood exhibited the opposite reaction. The relationship is therefore not clear as yet, although we hope that further investigation will solve this important problem.

Although at first glance nothing would seem more remote than the possibility of a connecting link between the opposing reactions of Groups A and B, careful analysis of the data and the three charts will reveal many points of similarity between them. A striking characteristic in each case is the irregularity of all the curves. The elevation or depression does not proceed evenly upward or downward, but rather by a series of irregular, tidelike fluctuations, until the peak of leucocytosis or the trough of leucopenia is reached. Another important similarity is the late period in which the maximum leucocytosis or leucopenia develops. This fact emphasizes the necessity of making frequently repeated observations over long periods of time. Again, in Group A the leucocytosis is due primarily to an increase in neutrophilic polymorphonuclear leucocytes, the lymphocytes being at first depressed and increasing relatively later. In Group B the leucopenia is also chiefly polymorphonuclear in nature, the lymphocytes being relatively unaffected. We cannot resist the opportunity, at this point, to call attention to the many points of similarity between this type of neutrophilic leucopenia and the leucopenia of malaria, and to suggest that *blood destruction is in both cases the common factor which may be responsible for that reaction.* We hope to develop this conception more fully in our forthcoming report on autohemopyretotherapy.

We have spoken of the various characteristics that Groups A and B possess either separately or in common. But the one that has piqued our curiosity more than any other still remains to be discussed. We refer to the mirror image relationship between the total leucocyte and, even more strikingly, between the polymorphonuclear curves of the two groups. In our opinion, this phenomenon, more than any other, establishes definitely the specific nature of our results.

We realize that seven patients do not form a very imposing array from which one might be tempted to draw final conclusions, but, on the other hand, they have been so painstakingly investigated and have yielded such significant data that the subject seems to be worthy of considerable additional investigation. This we are planning to undertake. Meanwhile, it is hoped that this preliminary report, by calling attention to the many interesting problems involved, may lend added impetus to the experimental study, not only of the newly conceived autohemopyretotherapy, but also autohemotherapy in general. Finally, in closing our discussion, we would like to make this plea. Let those who are interested in elevating autohemotherapy to the rank that it merits devote attention not alone to its therapeutic manifestations, but also to the scientific mechanism underlying them, for one must depend primarily upon the experimental approach if it is desired to establish autohemotherapy upon a permanently sound foundation.

SUMMARY

1. A simple method is described for the intravenous injection of hemolyzed, citrated, autogenous blood. The use of this technique makes possible the injection of relatively large amounts of blood, without ill effects.

2. According to the nature of the leucocytic reaction following the injection, individuals are found to fall into two groups: Group A exhibits leucocytosis; Group B, leucopenia. Separate characteristics and points of similarity between the two groups are pointed out, the most striking being the fact that the curves of the total leucocytes and polymorphonuclear cells of each group, although opposed in direction, are, nevertheless, mirror images of each other. These phenomena are taken to indicate the specific nature of the results obtained.

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CHANGES IN ERYTHROCYTE FRAGILITY DUE TO PHYSICAL EXERCISE AND VARIATION OF BODY TEMPERATURE*

JOHN EMERSON DAVIS, PH.D., UNIVERSITY, ALA.

IN 1921 Hastings¹ showed that the resistance of erythrocytes to laking by hypotonic salt solutions is increased in dogs immediately after a period of treadmill running. Lieberman and Acél² reported similar findings in rabbits, guinea pigs, and men, with the exception that they observed a decreased erythrocyte resistance for ten minutes immediately after the exercise period.

Thörner³ reported an increase of erythrocyte osmotic resistance in dogs as a chronic effect of daily physical training by treadmill running. Davis and Brewer⁴ reported a decreased erythrocyte resistance in one dog trained by treadmill running, and an increased erythrocyte osmotic resistance in two dogs trained by daily swimming in water at 30° C. These workers suggested that the body temperature during exercise may be an important determining factor.

One purpose of this investigation was to obtain more data upon the chronic effect of treadmill running on cell resistance in dogs. Another objective was to

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investigate a possible relationship between body temperature and erythrocyte osmotic resistance

PROCEDURE

Five dogs were confined in a large cage and placed on a constant diet of Purina dog food for two weeks before control determinations on the blood were made. Erythrocyte fragility determinations were then made on each dog by Hasting's method¹ at various times over periods of from one to two and one half weeks. Erythrocyte numbers and hemoglobin percentages (Sahli) were also determined. The blood was drawn from the saphenous vein, after the dog had

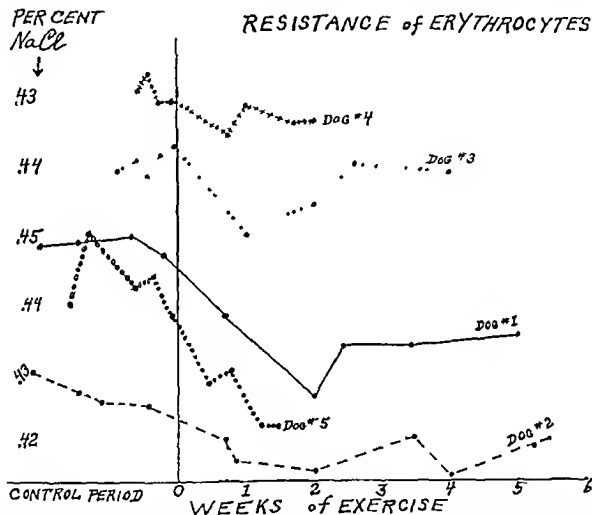


Fig. 1—The chronic effect of daily treadmill running upon the osmotic resistance of erythrocytes in five dogs. The resistance is expressed as the percentage of sodium chloride solution that causes 50 per cent hemolysis.

rested quietly (blinded) on a table for twenty minutes, and was also in a basal state with respect to previous exercise and food.

The daily exercise during the training period consisted of running a distance of 5 miles in one hour on a treadmill inclined at a 25 per cent grade. A few minutes of rest was always allowed the dogs after the first half hour of running.

In later studies designed to investigate the influence of body temperature upon red cell fragility, dogs were immersed in a tank of water for a period of thirty minutes. The water in the tank was maintained at 43° C in experiments intended to raise the body temperature, and at 22° C when it was desired to reduce the rectal temperature.

RESULTS

Chronic Results.—In all five dogs, the erythrocytes became less resistant osmotically during the first week of treadmill running. This result is not in agreement with the work of Thörner³ (Fig. 1). The resistance is expressed in terms of the concentration of sodium chloride which caused 50 per cent hemolysis in one hour. Dog No. 4 died of pneumonia after losing weight rapidly, but all of the other animals appeared to be exceedingly healthy and lost no weight throughout the experimental periods.

During the first week of regular exercise, the hemoglobin per unit volume of blood was reduced in all dogs by 10 to 18 per cent of the preexercise normal value, and the erythrocyte numbers were reduced correspondingly. After 3.5 weeks of training, the erythrocyte numbers of Dogs 1 and 2 exceeded the preexercise normal value by 20 and 14 per cent, respectively. These findings confirm previous work by Davis and Brewer.⁴

It was suspected that the apparent fragility of erythrocytes might be related to changes in the osmotic pressure of the blood serum due to regular daily exercise. Therefore, the freezing point depression of the serum was determined occasionally in two of the dogs on blood from the same samples which furnished material for fragility tests. These determinations were made with a Heidenhain thermometer on 5 c.c. samples of blood serum. No correlation was found in these experiments between the erythrocyte osmotic resistance and the serum osmotic pressure.

TABLE I

AVERAGE CHANGES IN THE BLOOD OF TWO DOGS IMMEDIATELY AFTER TREADMILL EXERCISE

	DOG NO. 3		DOG NO. 2	
	BEFORE EXERCISE	AFTER 20 MINUTES RUN	BEFORE EXERCISE	AFTER 30 MINUTES RUN
Erythrocyte number	4,800,000	5,570,000	5,770,000	7,440,000
Hemoglobin per cent (Sahli)	67	79	85	100
Hematocrit cell volume	27 per cent	32.5 per cent	34 per cent	43.5 per cent
Body temperature (Fahrenheit)	101.3°	105.4°	100.8°	105.1°
Serum osmotic pressure (Δ)	0.526	0.551	0.547	0.557
R.B.C. Fragility as per cent NaCl for 50 per cent laking	0.440	0.414	0.471	0.453

Results Immediately After Exercise.—Table I is presented to show changes in body temperature, erythrocyte fragility, and serum osmotic pressure that were observed immediately after exercise. All values given for each dog are the average of three experiments. In all experiments all of the values for each determination changed in the same direction, except in one experiment on Dog No. 2 in which the serum osmotic pressure was observed to be diminished after exercise. The increased osmotic resistance reported here confirms the work of Hastings¹ on the dog, and the older work of Manea⁵ (1896), cited by Hastings,¹ for human beings. Hastings found an increased chloride content of the plasma, and a decreased chloride content of the erythrocytes after exercise, and suggested the possibility that the osmotic pressure of the cell contents may be diminished.

TABLE II
EFFECT OF HOT AND COLD BATHS ON THE DOG

	HOT BATH IN WATER AT 43° C FOR 30 MINUTES			COLD BATH IN WATER AT 23° C FOR 30 MINUTES		
	BEFORE	IMMEDIATELY AFTER	24 HOURS AFTER	BEFORE	IMMEDIATELY AFTER	24 HOURS AFTER
Erythrocyte number	5 190 000	6,310 000	--	4 930 000	6,920 000	4 860 000
Hemoglobin per cent (Sahli)	72	95	--	68	91	75
Hematocrit	37 per cent	34 per cent	--	32 per cent	40 per cent	35 per cent
Body temperature (rectal)	101.9°	104.4°	101.8°	101.3°	99.6°	101.0°
RBC Fragility as per cent NaCl causing 50 per cent hemolysis	0.427	0.417	0.437	0.444	0.464	0.430

Hot and Cold Baths—Table II shows typical experiments in which dogs were given hot and cold baths of thirty minutes' duration. Immediately after a hot bath, the osmotic resistance of red cells is increased, but twenty four hours later it is decreased to a value below the normal. The increase of rectal temperature observed immediately after the bath was about 2.5° F. This was not so great as that observed after exercise but the increase in erythrocyte resistance was also correspondingly less. Cold baths caused just the opposite changes in resistance, namely, a decrease immediately after, and an increase above normal twenty four hours after the bath. The body temperature was lowered by about 1.7° F. by the cold baths. Practically no exercise was involved in these baths.

No correlation was found to exist between occasionally determined serum osmotic pressure and erythrocyte fragility after the baths.

DISCUSSION

I believe that the subnormal cell resistance observed twenty four hours after a hot bath is caused by the same factors which produce a diminution of resistance during a period of physical training by treadmill running, and that the elevated body temperature during running or hot bathing is primarily responsible for the observed changes in red blood cell resistance. Similarly cold baths seem to affect the cell resistance to laking in the same manner as exercise by swimming in cool water, as regards the chronic or delayed effect previously reported by Davis and Brewer.⁴

Rice and Steinhaus⁶ have shown that treadmill running produces in dogs an increase in the pH of the blood, and that this is due to overheating, over ventilation, and blowing off of carbon dioxide by the lungs. Then dogs were run for about one half an hour at a speed of 5 miles per hour on a treadmill inclined at 25 per cent grade. These exercise conditions are almost identical with those which prevailed in my experiments given in Table I. Since I did not attempt to determine the pH of the blood, I have not precluded the possibility that this is a determining factor in red corpuscle resistance changes due to physical exercise.

CONCLUSIONS

Daily treadmill running produces in dogs a decrease in the (resting) resistance of erythrocytes to laking by hypotonic salt solutions during the first week of training. The erythrocyte osmotic resistance, as determined *immediately* after running exercise, is increased.

The body temperature during exercise is probably a primary factor in causing this chronic decrease due to daily running exercise as well as the previously reported increased resistance produced by daily swimming in water at 25° to 30° C. Hot baths caused the same changes of erythrocyte fragility as treadmill running, while cold baths produced the same chronic change as swimming.

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THE EXCRETION OF SULFANILAMIDE IN PERSPIRATION*

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THE measurement of the sulfanilamide excreted in urine has been proposed by Marshall¹ as a method by which the dosage of prontosil might be measured and by which the efficacy of that dosage might be estimated. The low excretion of this drug in the urine of one of our patients during an extreme hot spell led us to extract his clothing and to test the extract qualitatively for sulfanilamide. We found that appreciable amounts of this compound were recovered from the perspiration contained in the clothes. The clothes of five subsequent patients on this medication were extracted. Prontosil was recovered in appreciable quantities in each case. We feel, therefore, that in hot weather, the excretion of para-amino-benzenesulfonamide in perspiration may lead to its lowered excretion in the urine.

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*From Department of Pediatrics, University of Chicago.
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DEGENERATIVE LEUCOCYTIC TRANSFORMATIONS ASSOCIATED WITH AGING*

DIFFERENTIAL IDENTIFICATION OF MONOCYTES

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THE life span of human white blood cells in the peripheral blood stream varies from a few hours to approximately four days^{1,4}. White blood cells in sealed (vaseline) cover slip preparations have a similar life span. Such preparations with supravital stains⁵ offer a means for studying viable cells. Together with other factors, the viability of white blood cells is determined by the cell motility, the movement of granules and vacuoles within the cytoplasm, the size of the vacuoles, the Brownian motion, and changes in the staining characteristics of these intracellular structures. In the original technique of Sabin both neutral red and Janus green are employed for staining purposes. The former is only slightly toxic to cells,^{6,8} whereas the latter does not permit the cells to survive more than three to four hours and, therefore, is not satisfactory for prolonged studies. In view of this fact, we employed the Sabin technique with the exclusion of Janus green to study the life span of white blood cells at room temperature.

Of the many changes which occur during the life span of a white blood cell *in vitro*, those associated with the vacuoles are most interesting, the process of autolysis being the final stage. Vacuoles take up the neutral red stain and thereby become readily visible. It is believed by some that vacuoles are cytoplasmic invaginations in which digestive processes take place, and that the enzymes produced for these digestive processes absorb the neutral red.^{9,10} White blood cells, when grown on tissue culture media, develop vacuoles with age.¹¹ We have observed that this also occurs in the neutral red supravital preparations and that these vacuolar changes are chiefly responsible for the morphologic alterations of the cells during cellular degeneration.

The majority of white blood cells of human peripheral blood develop vacuoles in these supravital preparations. Among other changes we have observed specific vacuolar alterations in the monocytes. These are quite uniform and constant in their development. During the first ten to twenty minutes of staining the monocytic vacuoles are small (size of neutrophilic granules) of salmon pink color, and often arranged in a rosette. For the next two to three hours they enlarge gradually until a nearly uniform ovoid shape of about one micron

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in diameter is seen. At this stage we find the cytoplasm of the monocyte filled with uniformly stained vacuoles; the cell itself becomes nonmobile and assumes a spheroid shape. Such monocytes resemble macrophages because of the numerous vacuoles which are common to the latter. The development of the vacuoles during the first two to three hours may be due to some process associated with

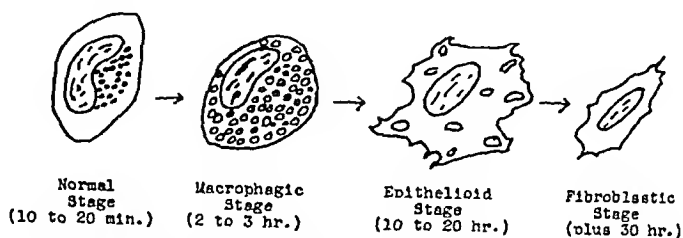


Fig. 1.—Diagrammatic presentation of sequence of degenerative changes observed in neutral red supravital preparations of monocytes. See text

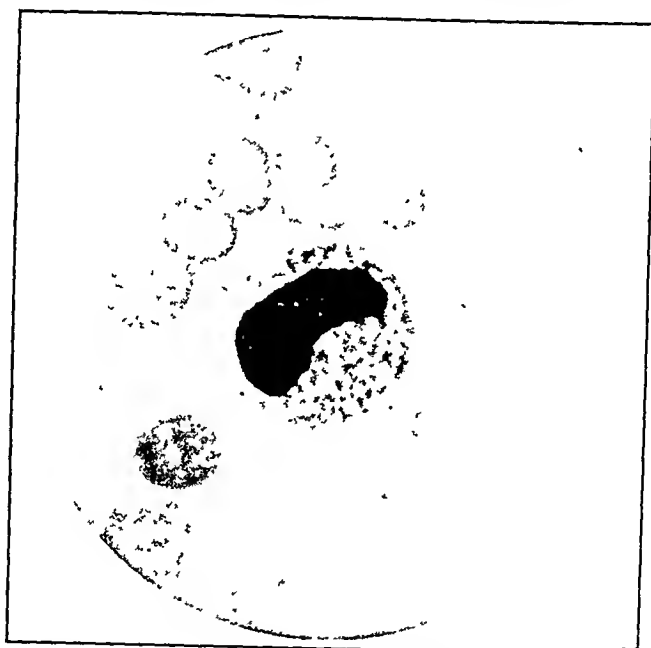


Fig. 2.—The "macrophagic" stage of a normal human monocyte from a three-hour-old cover slip preparation after fixation and staining by the Jenner-Giemsa technique ($\times 2000$). See text.

starvation, since Koehring observed that paramecia grown on rich media developed few vacuoles, whereas on nutritionally inadequate media vacuolar development was marked.

After this "macrophagic" stage is maintained for a number of hours (five to twenty-four hours), the monocytes begin to degenerate and the vacuoles continue to expand and subsequently rupture and lose their red-stained contents. Some of the monocytes become a degenerated colorless mass within forty-eight hours while others partially maintain their cytoplasmic continuity and enlarge, assuming an appearance similar to epithelioid cells. Later, when the cytoplasm

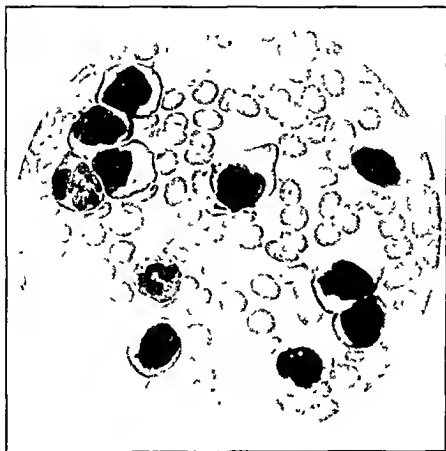
Fig 3A ($\times 1000$)Fig 3B ($\times 2000$)

Fig 3—Blood from a case of monocytoid myeloblastic leucemia illustrating the effect of aging in a neutral red supravital preparation. A, before the preparation and B, five hours after preparation, showing the "macrophagic" state of vacuolar degeneration.

becomes frayed, with all of the vacuoles ruptured, some cells assume an appearance similar to that of fibroblasts. Apparently only the very viable cells go through all of the morphologic stages described above. The sequence of events is illustrated diagrammatically in Fig. 1.

It is possible to obtain permanent records of any of these stages by gently removing the cover slip and then fixing and staining the blood smear. This is illustrated by Fig. 2, which demonstrates a Jenner-Giemsa stained cell at the three-hour stage when vacuolated monocytes resemble macrophages in appearance.

The above-mentioned observations become of practical interest when it is difficult to differentiate between monocytes and promyelocytes. The development of numerous vacuoles in the monocytes in a neutral red supravital preparation offers a definite criterion for the identification of these cells. However, if the cells under question do not show uniform vacuolization within three hours, they are in all probability myelocytes.

As we have already stated, vacuolization is common to most white cells on aging, but the extent of this process is not so characteristic in other cells as it is for the monocytes. The fact that different white cells develop numerous vacuoles and thereby approach macrophages has given rise to the concept that macrophages may originate from other cells.

Awrorow and Timofejewskij¹² concluded that myeloblasts and lymphocytes were changed to macrophages when leucemic blood was cultured. We have observed similar changes with the neutral red supravital technique (Fig. 3). From the morphologic point of view it is difficult to distinguish a true macrophage from the "macrophagic" stage of various degenerated cells. However, whereas the true macrophage is actively phagocytic for debris and fragmented red cells, the "macrophagic" cells do not exhibit a similar functional activity.

In addition to the above it is believed by many that lymphocytes develop into macrophages,¹³⁻¹⁵ that reticulum cells develop into macrophages,¹⁶ that monocytes are transformed into macrophages and subsequently into epithelial cells,¹⁷⁻²⁰ and that both myeloid and lymphatic leucemia blood cells²¹ change to vacuolated "macrophagic" cells. With the neutral red supravital technique the development of the "macrophagic" stages in lymphocytes, reticulum cells, monocytes, lymphoblasts, and myeloblasts can be observed.

Although polymorphonuclear leucocytes do not usually undergo appreciable changes in neutral red supravital preparations, it is possible to observe the "macrophagic" stage even in these cells. This may be exemplified by the changes observed in the polymorphonuclear leucocytes in septic tissue sites (Fig. 4).

The fact that so many different types of cells assume a similar appearance during aging, but do not reveal the specific functional aspects of macrophages, is indicative of the possibility that these cells do not become true macrophages but are merely passing through one aspect of the degenerative process that we have described above.

It seems to us that if true macrophages can develop from monocytes, one should find many more macrophages in tuberculosis, leucopenic infectious monocyctosis, undulant fever, Hodgkin's disease, etc., in which monocytes are abnormally increased. Furthermore, it might be expected that in such instances in which macrophages are found in increased numbers (malaria, kala azar,²² subacute bacterial endocarditis, and syphilis²³), an associated or proceeding monocyctosis would be observed. Likewise, there is no increase in the number of macrophages following the monocyctosis of the blood²⁴ of human subjects subjected to tuberculin injections.

These considerations lend emphasis to our observations that the development of cells resembling macrophages is not indicative of a functional transformation but is merely a degenerative phenomena associated with aging of the



Fig. 4.—Polymorphonuclears and monocytes obtained from the pus of a furuncle. These cells through vacuolated degeneration have developed a macrophage like appearance.

cells irrespective of whether this process occurs *in vivo* as a development of so called macrophages in pleural, abdominal, and other cavities or *in vitro* as in the neutral red supravital technique.

SUMMARY AND CONCLUSIONS

1. Various stages of degeneration of white blood cells have been observed with the neutral red supravital technique (Fig. 1).
2. Monocytes can be identified by characteristic vacuolar changes (Fig. 2).
3. The development of a "macrophage" stage by various white cells during aging indicates that this is a degenerative process and not a transformation of one physiologic type to another.

The author wishes to express his appreciation to Dr. N. Rosenthal for his interest and aid.

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LEUCOCYTIC VARIABILITY AND ITS RELATION TO METEOROLOGIC ALTERATIONS*

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IN RECENT years many papers have appeared discussing leucocytic variability and the various factors that influence it. It is the purpose of this paper to discuss the influence of the meteorologic alterations on the leucocyte count.

In reviewing the literature, our attention was first called by Alvarez¹ to a paper on Filariasis which he wrote in which he noted changes in the eosinophilic lymphocyte level and variations in the neutrophil count associated with the cyclic changes occurring with the moon in Porto Rico. In a later personal communication he observed an increase in the leucocyte count and the polymorphonuclears associated with the period of low barometric pressure during the cyclone of St. Nicholas, which passed over Porto Rico on September 10, 11, and 12, 1931. Stetson (1927)² observed a seasonal variation in the polymorphonuclear cells, with the highest average percentage in June and the lowest average percentage in December. On the other hand, the highest average percentage of lymphocytes was in December. He also found day to day fluctuations in the white blood count in which each type of cell participated. These fluctuations were beyond the limits, dependant upon technical or mathematical errors. Harvey and Hamilton (1934)³ also observed daily variations in the blood count. In one allergic individual these fluctuations were much greater than in the normal. Zim and Bauernmeister (1933)⁴ and others have also observed abrupt changes. Shaw (1934)⁵ observed a case in which he made leucocyte counts for the period of one year. These observations were made in Cairo, Egypt. He found a persistent left handed deflection, and no seasonal variations. He observed that the range of variation in the year is the same as in one person in twenty four hours but less than in a population. Kennedy and Mackay (1935)⁶ observed that the polymuclear count of healthy British annen in Iraq was not significantly different from the indigenous population and both groups were definitely deviated to the left as compared with health standards in Britain. Also pathologic cases and normal rabbit counts were more left handed than in Britain. The authors suggested that the most probable causative factor was climatic.

In the summer of 1932, we became interested in studying these daily fluctuations in the leucocyte count. We made daily counts under basal conditions at the same time every morning on four normal individuals. The pipettes and counting chamber were certified by the U. S. Bureau of Standards, and the same pipette was used throughout the study for each individual.

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Of our four normals, subject No. 1 was of a pyknic habitus; No. 2, a mixed type; No. 3, a leptosome; and No. 4, a leptosome of somewhat heavier proportions.

TABLE I

PATIENT NO.	AVERAGE COUNTS	
	SUMMER	WINTER
1	9,350	10,600
2	5,700	
3	7,790	8,460
4	4,590	

We observed that the average level of the count and the degree of fluctuation varied with each individual (see Table I). Subject 1, with the pyknic habitus, showed the highest level, while No. 4, a leptosome who was strongly sympathicotonic, showed the lowest average level; and Nos. 2 and 3 occupied intermediate positions. In comparing the summer levels (July and August) with the winter levels (December and January) we found that the average counts in winter were higher than those of the summer, while the degree of fluctuation was greater in the summer than in the winter. The individual with the pyknic habitus not only had the highest level, but also showed the greatest degree of fluctuations, while the strongly sympathicotonic individual not only showed the lowest level, but also showed the lowest degree of fluctuations.

Variations in levels with constitutional differences have been previously observed by Moewes⁸ and Isaacs,⁹ while fluctuations of the counts around various levels have been observed by Doan and Zerfas,¹⁰ Smith and McDowell,¹¹ Jackson and Stovall,¹² Shaw,¹³ and Harvey and Hamilton.³

The average levels of the differential counts may be observed in Table II. The neutrophile levels are slightly lower in winter in subject 1 and almost the same level in subject 3; however, both basophile and eosinophile levels are noticeably elevated in both in the winter.

TABLE II

	SUMMER				WINTER	
	1	2	3	4	1	3
Neutrophiles	66	67	66	55	61	64
Lymphocytes	31	30	32	46		
Eosinophiles	0.86	0.76	0.74	0.35	2.3	3.5
Basophiles	0.06		0.21	0.19	0.27	0.65
Monocytes	2.2	1.82	1.82	2.06		

Of particular interest are the daily leucocyte counts made during the summer and winter and the correlation of the counts with meteorological alterations.¹⁴ Chart 1 is presented, summarizing the results in subjects 1 and 3.

At the top of the chart is the barograph. The line at the top of the graph represents the wind velocity; the blocks of black and white, the percentage of sunshine and cloudiness; the heavy line, the barometric pressure; the dotted line, the temperature; and the vertical lines, the degree of weather instability. The associated peaks with the meteorologic episodes are lettered from A to Y.

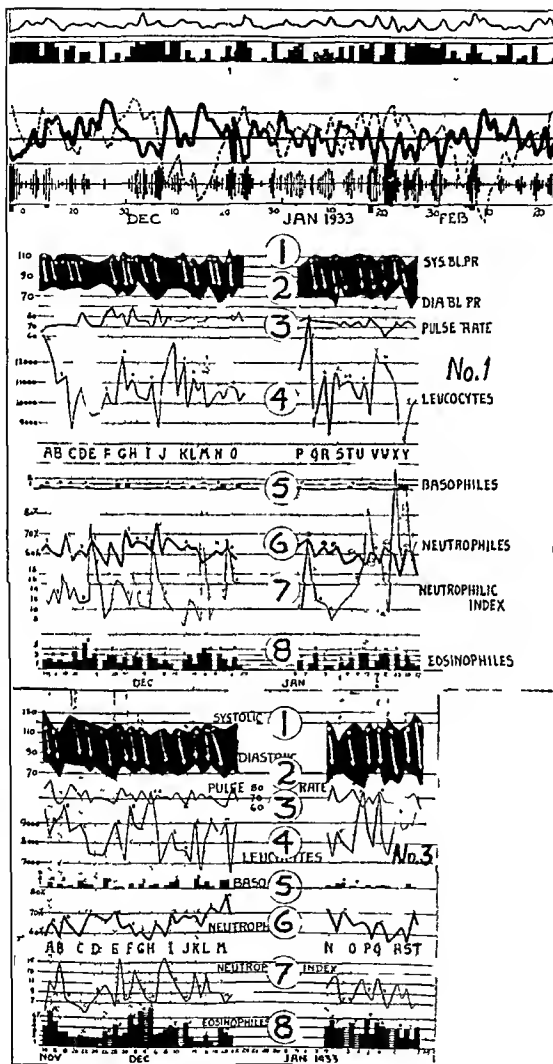


Chart 1

In viewing the graphs we note that following the polar infall (rising barometric pressure and falling temperature) of November 15 (episode 1) there is a slight rise in the eosinophiles, a fall in the nuclear index¹⁵ (more young forms), a rise in the neutrophiles and total leucocyte count, and a fall in the diastolic pressure. On November 21 (episode 4) there is a marked polar infall. This is associated (response D) with a rise in systolic blood pressure, followed by a fall in the diastolic pressure, a rise in the total leucocyte count and eosinophiles, and a fall in the nuclear index. A similar sequence of events of varying degrees is found with most of the meteorologic episodes.

In January frequent polar infalls occur with marked changes. In general, the more pronounced the meteorological, the more pronounced the peaks in the leucocyte picture. The degree and promptness of the response varied with the individual.

The meteorologic alterations have been shown for the most part to be associated with pressor episodes. Following these, there then occurs a vascular dilatation with a fall in diastolic pressure which is associated with an increase in the eosinophiles, the appearance of more young forms, and increase in the neutrophiles and in the total leucocyte count. The fluctuations may in part be explained by the emptying of the reservoirs of cells, such as the spleen, liver, and lungs.¹⁶⁻¹⁸ Meyer, Seevers, and Beatty¹⁸ observed a temporary leucocytosis in rats following a reduction in barometric pressure. Elias and Kaunitz¹⁹ had previously observed that reduced atmospheric pressure producing an anoxemia resulted in a leucopoiesis. Alterations in the splanchno-peripheral balance,^{20, 21} with splanchnic dilatation and peripheral vasoconstriction and vice versa, probably play a rôle.

Besides these factors of release of leucocytes from the bone marrow, redistribution of cells, and alterations of splanchno-peripheral balance, the stimulation of the bone marrow by the relative anoxemia resulting from the pressor episodes must be considered.¹⁴ Elias and Kaunitz¹⁹ have demonstrated the influence of anoxemia causing a leucopoiesis, and an associated hunger increased the response while feeding carbohydrates decreased the level evidently by counteracting the acidosis. Murakami²² also observed the influence of acidosis on increasing the polymorphonuclear leucocytes. Bier,²³ Freeman,²⁴ and others have observed the influence of anoxemia and the increase in tissue metabolites in stimulating flow of blood. In our group of normals at the onset of a polar infall, there is a relative leucopenia with a diminished number of neutrophiles, as well as an increase in systolic pressure; however, at the crest of the cold front and during the development of a tropical wave, the systolic pressure is generally lowered and the diastolic pressure falls (anoxemic phase¹⁴) and an increase in the number of eosinophiles and young forms, an increased frequency of appearance of basophiles, as well as an increase in the total count prevails. During the winter, with the frequent passages of the cyclonic interfaces, with pronounced swings in cold and warm weather, a greater stimulation is evident in the higher leucocytic level, the increase in eosinophiles, and the higher levels of fluctuation.

The leucocytic levels and the degrees of fluctuations are conditioned by the constitutional make-up of the individual.

A series of daily studies on groups of patients were carried out at various periods from 1933 to the present day. Because the volume of material would be too cumbersome to present, we have confined ourselves to the daily records of five of the patients.

In Chart 2 may be seen the record of patients J and G which was made during June and July of 1934. The record of J is placed beneath the barograph, while the record of G is at the bottom of the chart.

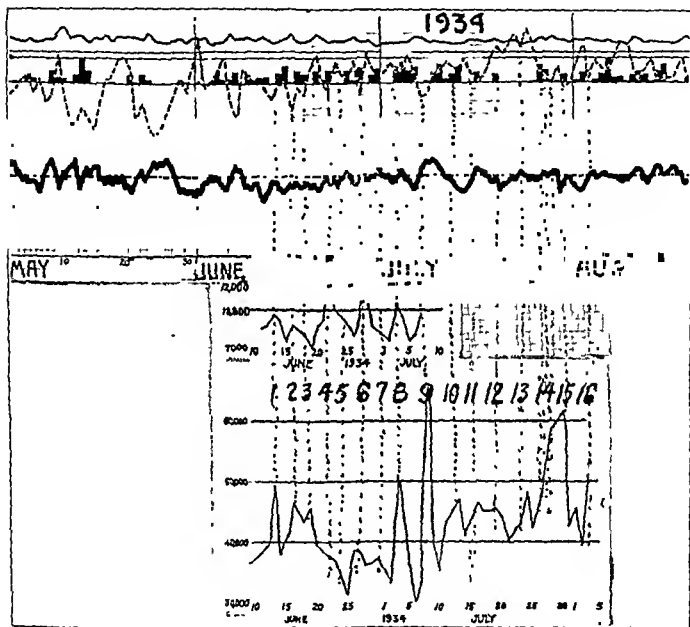


Chart 2

In looking over the chart of J (aleucemic lymphadenosis) we observe that nine polar episodes occurred and with seven of these peaks in the leucocyte count are associated (77 per cent). The peaks are slender with reversal taking place rapidly after the rise to reach a rather uniform base line.

On the other hand, the record of G (myelogenous leucemia) shows wide fluctuations. With fourteen of the sixteen polar episodes that occurred, leucocytic peaks are found (87 per cent). In this case we see the pronounced redistribution of cells as well as stimulation of bone marrow which probably occurs

with the polar episodes. This is a different picture than is seen in the case of lymphatic leucemia (see patient Cr). In comparing the peaks of J and G, a synchronism which is very close is observed, although the characters of the peaks are different.

The six-month record of a patient (R) with chronic far-advanced pulmonary tuberculosis reveals the following:

For the most part the peaks in the total leucocyte count occur with peaks in the neutrophiles; at times they occur with lymphocytic increases. A day or two after a peak in the immature cells, a peak in the neutrophiles occurs. This rhythm in shifting to the right in this patient occurs regularly almost without exception. The peaks in the neutrophiles and total leucocytes are not alone the result of the increase in immature forms, but the redistribution of cells, hydration, and other factors play a rôle. The eosinophile level is low and the cells appear at irregular times. Swings in the total leucocyte count do not occur with as marked rapidity as are seen in many of the other cases.

During the autumn the general level of the total leucocytes is higher and the fluctuations are greater than during the winter.

The per cent of immature neutrophiles is greater in the autumn (average 17 per cent) than in the winter (average 12 per cent), while the fluctuations are greater in the first period.

During the autumn, the better response in the immature cells and increased level of leucocytes are probably due in a good measure to the greater activity of the bone marrow.

The record of another patient (H) with far-advanced pulmonary tuberculosis revealed a similar daily record to the previous case. Increases in the leucocytes are associated with most of the polar episodes (73 per cent).

In the daily study of a case of chronic lymphatic leucemia, we found that the swings in the leucocyte count are sharp and abrupt. It is very interesting to note that the majority of the polar infalls are associated with depressed levels in the leucocyte counts, while the peaks occur after the polar episodes. This, as has been pointed out, is quite the opposite of what transpires in the patient with myelogenous leucemia.

SUMMARY

Alterations in the meteorologic environment bring about comparable changes in the human subjects under our observation. In our normals a period of increased systolic and diastolic pressure (vascular spasm) is followed by an increase in eosinophiles, the appearance of more immature forms, a rise in the polymorphonuclear leucocytes and total white count. The level and the degree of fluctuations are in a measure conditioned by the constitutional habitus of the individual. Seasonal variations are observed.

In our group of patients variations in the leucocyte count occurred with the meteorologic episodes; the degree and character of the fluctuations are influenced to varying degrees by the pathologic condition of the patient. Summaries of the daily records of five of our group of patients are presented.

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FORMATION OF ABNORMAL BLOOD PIGMENT AS A COMPLICATION OF SULFANILAMIDE THERAPY*

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IN THE past few years chemotherapeutic measures against acute infections have become increasingly important. Among such, sulfanilamide is outstanding. That it produces cyanosis is recognized. Our experience with the causative agent presents several interesting features.

A mild degree of cyanosis is often seen to develop on administration of sulfanilamide and to persist without much change during the course of the therapy. Obvious cyanosis noted early in the treatment may noticeably diminish despite continuance of the drug, although its disappearance is conditioned by withdrawal of sulfanilamide. On the other hand, the cyanosis may become sufficiently intense to assume clinical importance. Four such cases are presented herewith, the causative agent being subjected to chemical scrutiny.

CASE 1.—Female, aged 33 years, with ulcerative colitis confirmed by sigmoidoscopy and stool examinations; on culture hemolytic and nonhemolytic streptococci predominated, negative repeatedly for *E. histolytica*, *B. dysenteriae*, and tubercle bacillus. Table I indicates the various therapeutic measures and their relationship to cyanosis.

CASE 2.—Male, 3 years of age, with bilateral cervical adenitis; blood culture positive for hemolytic streptococci, likewise pus evacuated from incised swelling on ankle; pneumococci obtained on incision of both eardrums. The relatively large dose of sulfanilamide required to produce cyanosis is shown in Table I together with other therapy employed.

CASE 3.—Male, 31 years of age, with sinusitis; culture of exudate from punctured antrum positive for hemolytic streptococci. Cyanosis and dyspnea appeared within eight hours of first dose of sulfanilamide (1.2 grams) and one hour after the second dose. Oxygen therapy did not relieve the cyanosis until the drug was discontinued. The cyanosis cleared completely with oxygen forty-four hours after the last dose of sulfanilamide. Other therapy is given in Table I.

CASE 4.—Male, aged 3½ years, with pneumonia with left empyema; thoracentesis yielded greenish pus containing hemolytic streptococci; excoriating serosanguineous nasal discharge on culture revealed *C. diphtheriae* (antitoxin administered); no further evidence of diphtheritic involvement. After 19.2 grams of sulfanilamide, cyanosis was evident. Coincidental respiratory embarrassment was greater than could be attributed to the pulmonary disease. An oxygen tent did not relieve the cyanosis. The course of treatment is given in Table I.

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TABLE I

CASE 1—ULCERATIVE COLITIS				CASE 2—HEMOLYTIC STREPTOCOCCIC SEPSIS				CASE 3—HEMOLYTIC STREPTOCOCCIC SINUSITIS				CASE 4—HEMOLYTIC STREPTOCOCCIC EMPYEMA										
ATROPINE SULFATE	SODIUM PNEUMORRBITAL	BISMUTH SUBCARBONATE	SULFANILAMIDE	CYANOSIS	SPECTROSCOPIC	ACETYL SALICYLIC ACID	SULFANILAMIDE	PROTOSIL	CODINE SULFATE	SULFATE	ACEPHENETHIDIN	CODINE SULFATE	NORPHINE SULFATE	SULFANILAMIDE	CYANOSIS	SPECTROSCOPIC	PNEUMORRBITAL	ACETYL SALICYLIC ACID	SULFANILAMIDE	CYANOSIS	SPECTROSCOPIC	
1 0.001	0.25	320				0.3		0.75			1.5	0.48										
2 0.001	0.25	320				1.0		0.75	0.016		1.5	0.48										
3 0.001	0.25	320	0.3			0.6		0.37	0.016		1.5	0.48										
4 0.001	0.25	320	0.9			1.0		0.37	0.048		1.5	0.48										
5 0.001	0.25	320	0.9	+		0.6		0.37	0.048		1.5	0.48										
6 0.001	0.25	320	0.9	+		0.3		0.16	0.016		1.5	0.48										
7 0.001	0.25	320	0.9	+		1.0		0.48	0.048		1.5	0.48										
8 0.001	0.25	320	0.9		+	2.0					2.0	0.48	0.080	0.02								
9 0.001	0.25	320	0.9	+		2.0					2.0	0.48	0.065	0.02	1.2							
10 0.001	0.25	320	0.9	+		2.0					2.0	0.48	0.048	0.01	4.0							
11 0.001	0.25	320	0.9	+		2.0		0.010			2.0	0.48	0.048	0.01	4.0							
12 0.001	0.25	320	0.9	+		0.9					0.3		0.016	0.01	4.0							
13 0.001	0.25	320	0.9	+									0.065	0.065	2.6							
14													0.065									
15			0.3			1.3																
16			0.9			2.6																
17			0.9			2.6																
18			0.9			2.6																
19			0.9			2.6																
20			0.9			2.6																
21			2.0			2.6																
22			2.0			2.6																
23			2.0			2.6																
24			2.0			2.6																
25			2.0			2.6																

CHEMICAL DISCUSSION

In these patients, spectroscopic examination of the blood showed that the abnormal appearance of the specimens was associated with an absorption band in the red part of the spectrum. When the blood was diluted just enough to allow for the passage of light, this band divided the red-orange field into two parts occurring roughly in the ratio of 55:45, the line being slightly nearer the yellow end. On spectrometric measurement the density center of the absorption band, which was not sharply defined, was located at 634 millimicrons. This is the position given for methemoglobin by Bodansky.¹ Since Koch² places this band at 631 and Haurowitz³ at 625 millimicrons, the spectrometer was recalibrated by means of emission spectra and the position of the absorption bands of oxy-, carboxy-, met-, and sulf-hemoglobin verified.

Since at the time of our observations (January, 1937) the only abnormal pigment associated with sulfanilamide therapy was sulfhemoglobin, particular care was taken to ascertain the nature of the pigment encountered in our patients. That it was not sulfhemoglobin was demonstrated in several ways. Not only was the latter available for comparative study in a patient who had taken large and oft-repeated doses of acetanilide, but it was also prepared in the laboratory. Among the various bright lines used for calibrating the spectrometer was the broad band in the red given by calcium. This extends from 618 to 626 millimicrons which, in our hands, is precisely the position of the absorption spectrum of sulfhemoglobin. In recent investigations, Paton and Eaton⁴ report 615 to 628 millimicrons. According to Kahn and Goodridge,⁵ the characteristic range is from 610 to 625 millimicrons; Jean Prevot⁶ located it at 610-620, Haurowitz³ at 617 millimicrons. When this pigment was observed in a hand spectroscope under such conditions of dilution that only red and orange could be seen, the line divided the field into distinctly uneven parts, roughly in the ratio of 70:30, the narrower including the orange. Addition of ammonia or dilute ammonium sulfide did not affect the band produced by sulfhemoglobin, whereas it caused the disappearance of the band associated with sulfanilamide therapy. Likewise, 1 per cent solution of potassium cyanide caused the immediate disappearance of the line ascribed to methemoglobin, whereas it only slightly affected the sulfur derivative. Passage of illuminating gas through solutions containing sulfhemoglobin shifted the absorption band from 615 to 631 (maximum 622) to 611 to 624 (maximum 617) millimicrons. Clarke and Hurtle⁷ regarded this shift as evidence of a new compound, carboxysulfhemoglobin, and recorded the change in position of the absorption band as from 610-625 to 605-620 millimicrons. The line obtained with the pigment responsible for the cyanosis following sulfanilamide therapy disappeared on treatment with illuminating gas.

The response to illuminating gas is disconcerting since stability to carbon monoxide is regarded as one of the primary characteristics of the pigment. Nevertheless, dilution with water also caused the absorption band to disappear. Furthermore, the methemoglobin did not persist in the unhemolyzed sample, but slowly reverted to normal blood pigment. This was evidenced not

only by a change in the spectroscopic findings but also by disappearance of the typical "dirty" color of the blood sample. Because of this fading of the characteristic band, the spectroscopic examination of the blood was carried out immediately. In three of our cases the absorption band vanished completely within periods varying from five to fifty minutes once a suitable concentration for observation had been achieved. Excessive dilution was accompanied by fading within a few seconds. Addition of a few drops of the sample restored the band only to have it disappear again, though more slowly. In one instance, the absorption band persisted overnight. This behavior was noted by a varied group of curious observers who, without instruction or assistance, were asked to describe what they saw in the spectroscope.

The instability of methemoglobin is by no means a new idea. According to Stadie⁸ "methemoglobin disappears from the circulation with great rapidity whether it is introduced by injection of methemoglobin or formed within the circulation by the action of chemicals or of pneumococci." Harrop and Waterfield⁹ noted the "great instability of this pigment in the blood stream" and stated that "unless it is produced very rapidly it is destroyed before it is formed in appreciable amounts."

It should be emphasized that we have examined spectroscopically many specimens of blood from patients on sulfanilamide therapy who were slightly cyanotic without detecting abnormal pigment. Frequently, less than thirty seconds elapsed from withdrawal of the blood to its dilution and examination. A few samples were tested without dilution by allowing a drop of blood to fill a thin wedge shaped chamber formed by holding two microscope slides together with a wire clip, a fragment of broken slide separating them at one end; the hand spectroscope was applied to that part of the wedge which blotted out all but the red orange part of the spectrum.

Although it is probable that as little as 5 per cent saturation of the blood with methemoglobin will suffice for visual detection of darkening of the specimen, much larger concentrations are required for observation and measurement of its absorption band. According to Kobert (1902, cited by Peters and Van Slyke¹⁰) ordinary spectroscopic examination is uncertain when less than 25 per cent of the blood pigment is in the form of methemoglobin. Bloem,¹¹ in carefully controlled experiments, ascertained that the lower limit of detectability for methemoglobin was 0.3 gm per 100 cc of blood when diluted 1:5 and observed through tubes of 1 inch bore against a 60 watt frosted bulb. Bensley and Ross¹² found 0.4 to 0.9 gm per 100 cc to give a very faint band, over 2 gm of methemoglobin were required to obtain an absorption band designated as "one plus."

Although earlier investigators consistently reported sulfhemoglobin, more recent papers demonstrate the presence of methemoglobin. The literature repeatedly warns against the concurrent use of sulfates and sulfanilamide. Present evidence, however, is wholly inadequate to demonstrate that sulfhemoglobin is formed from sulfates or, for that matter, from sulfanilamide. Both compounds contain oxidized sulfur, whereas sulfhemoglobin is known

to be produced only in the presence of reduced sulfur. In the hemoglobin derivative the sulfur is negative; in these therapeutic agents sulfur functions as a positive element. As far as is known, sulfates are absorbed and excreted unchanged by the kidneys. Sulfanilamide as such has been shown to occur in urine.

Sulfhemoglobin is not even produced, ordinarily, by inhaling hydrogen sulfide because of the rapid oxidation to sulfate in the blood stream. Yet sulfhemoglobin is promptly formed when only traces of hydrogen sulfide are available once the normal blood pigment has been altered (presumably to methemoglobin) by certain drugs which are chemically related to aniline. A single large dose of acetanilide, for instance, may suffice to produce methemoglobin. Its continued use, however, leads to the formation of sulfhemoglobin, the necessary reduced sulfur being secured from the intestinal tract where hydrogen sulfide may have accumulated due to stasis or to an overabundance of sulfur-containing foods. A patient, therefore, who needs catharsis and fails to get it because of an interdiction against sulfates will still develop sulfhemoglobinemia provided the sulfanilamide "sensitizes" his blood pigment and his diet contains sulfur capable of yielding hydrogen sulfide.

It should be recognized that cyanosis associated with methemoglobinemia is temporary and responds promptly to withdrawal of the causative agent. Except in very mild cases, cyanosis occasioned by sulfhemoglobin persists for weeks since the pigment is extraordinarily stable both in the blood stream and in the laboratory. Although the cyanosis associated with sulfanilamide therapy may cause no clinical concern so long as it is due to methemoglobin, the possibility of the development of sulfhemoglobinemia should not be ignored. Sulfhemoglobin (never methemoglobin in our experience) may be detected by spectroscopic examination of a transilluminated ear lobe.

Because of the slight degree of cyanosis occurring in anemic patients with met- and sulf-hemoglobinemia, we wish to stress the warning sounded by Discombe.¹³ It is unusual for a patient with less than 5 gm. of hemoglobin per 100 c.c. of blood to become cyanotic.¹⁴ This is regarded as the "threshold value" for the appearance of cyanosis. Since it is the absolute level of non-oxygenated hemoglobin which determines the presence or absence of cyanosis, sulfanilamide administration must be undertaken cautiously when the total hemoglobin concentration is low. If the erythrocyte count is normal and *remains so* (twice weekly checking is advisable), the appearance of definite cyanosis is a satisfactory signal for temporary cessation of the therapy. With anemic patients, however, this warning is lacking. Spectroscopic examination may not suffice to indicate danger.

SUMMARY

1. Cyanosis may exist as a result of sulfanilamide therapy without the presence of abnormal pigment being revealed by spectroscopic examination.

2. The methemoglobin encountered in the cases described was highly unstable, thus interfering with detection and quantitative determination.

3 The abnormal pigment, although very unstable *in vivo*, persisted during oxygen administration but promptly disappeared upon discontinuance of the drug.

4 Some of the properties of met and sulf hemoglobin are described

CONCLUSION

The occurrence of cyanosis due to methemoglobin in four cases is presented as a specific effect of sulfanilamide therapy

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HYPERINDICANEMIA IN RENAL INSUFFICIENCY AND THE SIGNIFICANCE OF THE DIAZO REACTION*

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IN 1911 Obermeyer and Popper¹ demonstrated that indican was increased in the blood of uremic patients. Since then hyperindicanemia in renal insufficiency has been observed and confirmed by many investigators.²⁻¹³ From a review of this literature it is generally assumed that the normal range of plasma indican content is from 0.026 mg per cent to 0.086 mg per cent, although Haas,¹² in studying cases of intestinal putrefaction has shown that

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the plasma indican may rise as high as 0.15 mg. per cent and Rosenberg¹³ has demonstrated an increase as high as 7.0 mg. per cent in the plasma from cases in renal insufficiency.

In addition to the diagnostic and prognostic significance of hyperindicanemia, it has been suspected that indican might play a rôle in the production of uremia. This was emphasized when Harrison,¹⁴ Hewitt,¹⁵ Bolliger and Earlam^{7, 9} and others,¹⁶⁻¹⁹ pointed out that the diazo reaction of Andrewes¹⁷ occurred only in uremia, and Bolliger and Earlam⁷ claimed that the diazo reaction might be due to or dependent upon the indican content of plasma.

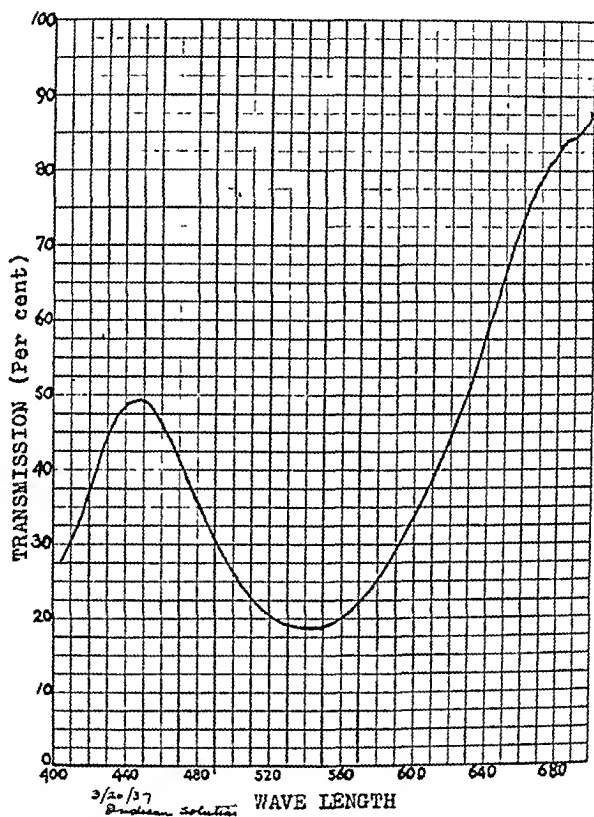


Fig. 1.—Spectrophotometric absorption curves of chromogen developed from indican in solution. A comparison of the curves obtained using blood plasma and urine show that all three are identical. These curves were obtained with the recording spectrophotometer of the Color Measurement Laboratory of the Massachusetts Institute of Technology.

The prognostic significance attached to the diazo reaction, therefore, might well be the same as that of hyperindicanemia. No great consistency, however, has been demonstrated in the results of past studies on indican, and the diazo reaction has been thought by some to be due, not to indican, but to such substances as the cyclic amines and the aromatic oxyacids.

In the light of such uncertainty it appeared that this subject deserved further consideration, and we, therefore, undertook to investigate the various methods of indican assay and the determination of the method most reliable.

Early in our work it was evident that Shalit's method²⁰ was specific for the determination of indican in the blood, as well as urine,²¹ and was more sensitive than the procedures more commonly used. Further evidence for this lies in the identity of spectrophotometric absorption curves of indican in solution (see Fig. 1) and of the chromogen developed from blood plasma and urine.

Shalit's results, while closer to the actual values than others, are somewhat inaccurate because of the use of cobalt sulfate ($\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$) as a standard for color comparison. Furthermore, the correction factor used in the original method could hardly give the desired accuracy. Nonspecific variables, such as glucose, play little or no part in the development of the color, except where low values are to be expected.

All workers in this field are aware of the fact that pure indican itself is not readily available and that errors may be derived from the use of standards other than indican. Moreover, if the plasma indican values in renal insufficiency are to be of any significance, one should have a method sensitive enough to measure the indican content in normal individuals where the amounts have been shown to be small. We have been able to demonstrate that indican is a normal constituent of blood present even in children as young as three months of age.

METHODS

Using the Evelyn photoelectric colorimeter -- Shalit's method was employed, using an appropriate filter transmitting the maximum amount of light at the wave length of $5,400 \mu\mu$. The choice of the filter for such a system was determined by the fact that the color developed has its maximum optical density at $5,400 \mu\mu$, as shown by the recording spectrophotometric absorption curves.

For purposes of convenience the method employed is presented in brief. The few changes that have been made from the procedure as described originally²² were necessitated for color measurement by the Evelyn photoelectric colorimeter.

Two cubic centimeters of plasma were diluted with 3 c.c. of distilled water and deproteinized by precipitation with 5 c.c. of 25 per cent trichloroacetic acid. To 5 c.c. of the clear filtrate (the aliquot being equivalent to 1 c.c. of plasma) in a calibrated centrifuge tube were added 5 drops of a 1 per cent solution of potassium persulfate and 0.5 c.c. of 1 per cent thymol in 95 per cent ethyl alcohol. This was shaken, and 5 c.c. of 12.5 per cent trichloroacetic acid in concentrated HCl were added. The solution was then corked inverted, and allowed to stand for fifteen minutes at room temperature, and then placed in a boiling water bath for twelve minutes. After standing again at room temperature for twenty-five minutes it was centrifuged for ten minutes. The supernatant fluid was carefully pipetted off, and the volume made up to 12 c.c. with glacial acetic acid. On stirring a perfectly transparent color developed.

TABLE I

CASES	INDICAN MG. %		B.U.N. MG. %		NONPROTEIN NITROGEN MG. %		INTRAVENOUS P.S.P. %		DIAZO REACTION
	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	
25 normals	0.26	0.08-0.50							
14 cases with various dis- eases without nephritis	0.38	0.21-0.90	16	11-28	33	29-37	-	-	0
13 hypertensives without nephritis	0.32	0.14-0.48	12	10-19	-	- - -	62	50-85	0
Nephrotic syndrome	0.22		29		41		50		0
Nephrotic syndrome	0.86		25		39		20		0
	1.14		32		-		15		0
Nephrosis	0.20		12		23		75		0
Subacute nephritis	1.24		44		65		0		0
Subacute nephritis	3.80		80		93		0		
	4.20		123		150		0		

Twenty-Six Cases of Chronic Glomerulonephritis

CASE No.	INDICAN MG. %	B.U.N. MG. %	N.P.N. MG. %	INTRAVENOUS P.S.P. %	DIAZO REACTION	REMARKS
6	0.14	--	28	--	Negative	
7	0.18	--	31	--	Negative	
8	0.18	11	--	75	Negative	
	0.40	13	--	70	Negative	
9	0.24	13	26	55	Negative	
10	0.24	54	--	20	Negative	
	2.50	155	--	0	Positive	Died
11	0.26	11	--	45	Negative	
12	0.28	15	--	65	Negative	
13	0.30	24	--	40	Negative	
	0.48	18	--	50	Negative	
14	0.30	18	--	--	Negative	
	0.56	22	31	60	Negative	
15	0.38	16	--	55	Negative	
16	0.40	8	--	50	Negative	
17	0.40	12	--	60	Negative	
18	0.40	17	--	55	Negative	
19	0.40	44	--	--	Negative	
	0.48	43	--	--	Negative	
20	0.58	13	--	40	Negative	
21	0.66	30	--	20	Negative	
	1.36	51	--	15	Negative	
22	0.92	27	46	50	Negative	
23	0.80	66	77	spt.	Negative	
	1.20	77	--	0	Negative	
24	1.60	83	--	spt.	Positive	
	3.20	115	--	0	Positive	Died
25	1.68	74	--	0	Positive	
	4.20	71	--	0	Positive	
26	2.80	123	--	spt.	Positive	Died
27	2.90	114	--	spt.	Positive	
	3.36	153	--	0	Positive	
	1.90	128	--	5	Positive	
	2.16	117	--	spt.	Positive	
28	3.40	113	--	0	Positive	
	4.30	126	--	0	Positive	
29	3.88	119	130	spt.	Positive	
	3.78	104	--	0	Positive	
30	4.40	200	--	0	Positive	Died
31	4.48	124	--	0	Positive	Died

TABLE I—CONT'D
Nine Cases of Chronic Vascular Nephritis

CASE NO	INDICAN MG %	BU \ MG %	NP \ MG %	INTRAVENOUS PSP %	DIAZO PEACTION	REMARKS
32	0.24	13	--	33	Negative	
33	0.60	44	60	7	Negative	
	1.16	48	66	0	Negative	
34	0.96	16	--	-	Negative	
35	1.00	33	--	20	Negative	
	1.04	25	--	-	Negative	
	0.82	42	--	-	Negative	
36	1.24	123	-	-	Negative	
	1.80	136	141	0	Positive	Died
37	1.40	33	-	10	Negative	Died
38	1.60	91	119	0	Positive	Died
39	1.40	81	--	2	Negative	
	2.60	95	--	0	Positive	
	2.38	105	-	0	Positive	
40	1.80	61	--	0	Positive	Died

Eight Cases With Chronic Vascular Nephritis and Cardiac Decompensation

41	0.20	15	--		Negative	Heart compensated
42	0.36	61	-	5	Negative	Decompensated
43	0.40	11	-	63	Negative	
44	0.62	25		15	Negative	Decompensation slight
45	0.74	--	94		Negative	Cerebral hemorrhage
	1.24	127	131	--	Negative	Died
46	0.74	25	-	28	Negative	Uncontrolled diabetes
47	1.06	82	110	0	Negative	Decompensated
	2.16	126	133	0	Positive	Died
48	1.80	72	-	0	Positive	
	1.20	50	-	5	Negative	

Six Cases With Other Renal Pathology

49	0.48	37	50	15	Negative	Hydronephrosis
	0.56	26		-	Negative	
50	0.34	18		-	Negative	Cancer of the prostate with acute retention
51	1.00	-	72	-	Negative	Enlarged prostate with acute retention relieved by operation
	0.60	-	41	-	Negative	
	0.36	-	34	-	Negative	
52	0.74	-	46	-	Negative	Left nephrectomy and right pyelonephritis
	0.80	-	54	-	Negative	
53	1.50	59	76	-	Positive	Pyelonephrosis in remaining kidney and cardiac decompensation
	1.60	64	-	0	Positive	
54	0.56	71	82	5	Negative	Acute cholecystitis and question of hepato renal syndrome
	0.90	36	60	10	Negative	
	0.34	15	36	32	Negative	

The solution was transferred to standard Evelyn colorimeter tube and placed in the colorimeter using a filter (Corning glass) transmitting between 5,200 $\mu\mu$ and 5,700 $\mu\mu$, with its maximum transmission at 5,400 $\mu\mu$. Readings were made from the galvanometer (1 mm to 0.027 microamperes) when the maximum deviation of the string had occurred. The result was read directly in milligrams per cent from a calibration curve previously obtained by plotting the galvanometer deflections from a series of known amounts of indican*.

*Indican was obtained from E. Merck Darmstadt, Germany, and from Dr. Fraenkel and Dr. Landau, Berlin, Germany.

used as standards, the amounts varying between 0.1 mg. per cent and 6.0 mg. per cent. The calibration curve obtained is shown in Fig. 2, the galvanometer readings (G) being plotted against milligrams indican per 100 c.c. of blood. This curve when plotted on semilogarithmic paper is a straight line, hence the value of X (milligrams indican) can be calculated by the following formula:

$$X = \frac{(2 - \log. G)}{K_2} \times 100 \text{ where } K_2 = 15.2,$$

provided the various volumes stated in the method are adhered to.

The patients studied comprised a large group from the Renal Vascular Clinic of the Peter Bent Brigham Hospital. All the patients had been under observation for long periods of time and their status was well known. The patients who were in a more advanced stage of their disease or in a critical condition, were for the most part from the wards of the hospital and in some cases the diagnosis was confirmed by postmortem examination.

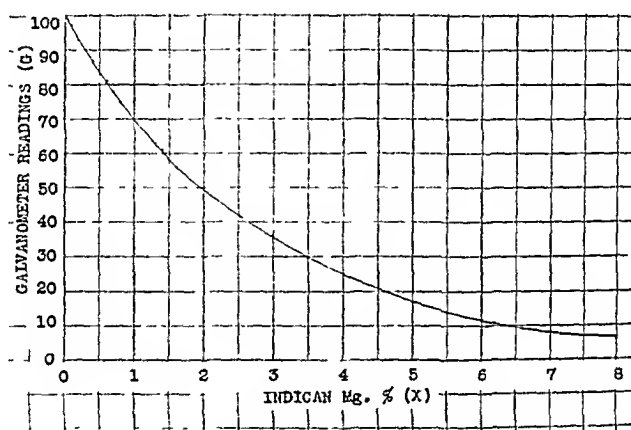


Fig. 2.—Calibration curve for the determination of indican in blood plasma in milligrams per cent (aliquot of indican solution equivalent to 2 c.c. blood plasma).

RESULTS

One hundred and six patients were studied with 164 determinations of the plasma indican content. The majority of the patients excluding 25 normals, used as controls, had parallel determinations of blood urea nitrogen or nonprotein nitrogen, phenolsulphonephthalein excretion, and diazo reaction in addition to the assay of the plasma indican, for purposes of correlation. The results are condensed in Table I.

The 25 normals showed an average plasma indican of 0.26 mg. per cent, with a range from 0.08 mg. per cent to 0.50 mg. per cent, which is considerably higher than values that have been usually quoted, confirming Sharlit's contention that the plasma indican content is higher than commonly assumed. In 14 cases with miscellaneous disease including syphilis, pernicious anemia, cirrhosis of the liver, bronchopneumonia, gastric ulcer, nonspecific enteritis, uncontrolled diabetes, cancer of the liver, and one case of subarachnoid hem-

orrhage, the average was 0.38 mg per cent, with a range from 0.21 mg per cent to 0.90 mg per cent. The highest indicans in this group were in a patient with untreated pernicious anemia and in a moribund man with carcinoma of the liver. In these cases the plasma indican was 0.60 mg per cent and 0.90 mg per cent, respectively. All of these patients had normal blood urea nitrogen values, except one with subarachnoid hemorrhage whose BUN was 28 mg per cent, curiously his indican was within normal limits. None of these patients had a positive diazo reaction.

Another group of 13 cases of vascular hypertension showed a plasma indican average of 0.32 mg per cent, an average blood urea nitrogen of 12 mg per cent, a PSP excretion of 62 per cent, and negative diazo reactions.

The most interesting group consisted of 43 cases of nephritis of various types as shown in Table I. In this group it will be noted that the plasma indican is elevated above normal limits or above the values found in the various unrelated diseases when there is also some elevation of the blood urea

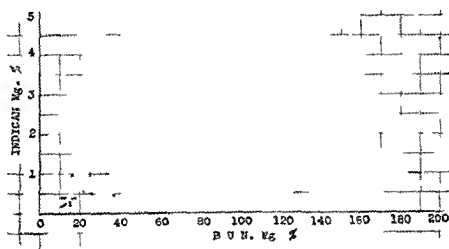


Fig. 3—Figure showing the relationship of BUN milligrams per cent to indican milligrams per cent

nitrogen and some impairment of the phenolsulphonephthalein excretion. The lowest value of the plasma indican was 0.14 mg per cent and 4 cases with values above 4.0 mg per cent, the highest determination being 4.48 mg per cent.

In practically all the cases with an increased blood urea nitrogen the plasma indican was elevated, although there is no direct relationship as shown in Fig. 3. Similarly, the increase in the plasma indican was usually associated with an impaired phenolsulphonephthalein excretion, but here the relationship was even less definite, due mainly to the inherent errors associated with phenolsulphonephthalein studies.

Another point of importance is that all patients with a plasma indican of over 1.50 mg per cent exhibited a positive diazo reaction and that 10 of 16 patients with such a positive reaction died, while the other six are all in a critical state with a poor prognostic outlook.

In regard to symptomatology we could find no definite correlation between the onset of nausea, vomiting, muscular twitching, convulsions or coma, and the rise in the blood indican.

In a study of 6 patients with kidney involvement other than nephritis, the plasma indican was elevated when there were other signs of retention of metabolic products, but once the condition was relieved by surgery or other treatment, the indican level decreased to normal along with the return of adequate renal function.

Similarly, in another group of cases with chronic vascular nephritis complicated with cardiac decompensation it was found that when renal insufficiency existed the blood indican was elevated to moderately high levels. With restoration of cardiac compensation, the blood indican decreased to a level consistent with the degree of renal insufficiency. In one of these cases the diazo reaction became negative.

The fact that the diazo reaction, as performed by Hewitt,¹⁵ was positive in those cases with an indican content above 1.50 mg. per cent was interesting and suggestive. It has been said that the diazo reaction is due to or dependent on the indican content of blood. To check this we made known solutions of indican in distilled water and in blood plasma in concentrations of 0.2 mg. per cent to 3.0 mg. per cent. The diazo reaction in these dilutions became positive at 1.5 mg. per cent. The pink color in this dilution was just perceptible and did not persist, but in a concentration of 1.8 mg. per cent the color was less fleeting and at 2.0 mg. per cent the color persisted for a definite interval.

We then submitted solutions of indican, indican in blood plasma, alcoholic extracts of urine and blood plasma in which the indican content was known to be high, also to the diazo reactions and secured spectrophotometric curves which definitely demonstrated that the colors developed were similar. It is difficult to obtain a satisfactory absorption curve of the diazo reaction in a sample where the reaction is not pronounced and also fades rapidly, but in those with an extremely high indican and consequently a more persistent diazo reaction, the absorption curves leave little doubt as to the similarity of the curves obtained to those in which known indican was added. From this we conclude that the development of color in the diazo reaction is dependent upon an increased indican content of the blood plasma of patients.

DISCUSSION

In the past numerous investigators have suggested that the determination of indican in blood was an excellent test of renal insufficiency and, in many cases, a more reliable index than blood urea nitrogen or nonprotein nitrogen. Interpretation of our data does not allow such a sweeping statement. Indican is increased in the blood of patients with renal insufficiency, but it is associated usually with an elevation of the blood urea nitrogen and a decreased phenolsulphonephthalein excretion. In a certain group of cases where extrarenal factors have given rise to renal impairment, the indican is temporarily increased, but with improvement in the patient, the indican returns to a lower level as do the other retained metabolic products, such as urea nitrogen. From the data obtained it appears that an elevation of indican above a level of 0.50 mg. per cent may be taken as an indication of renal

insufficiency. However, the determination of indican adds little if anything to the study of these patients from a diagnostic point of view.

The diazo reaction has already been set forth as a prognostic test in nephritis by other workers, and when positive, it is generally accepted that the prognosis is poor and in most cases fatal. In our limited experience this fact has been confirmed since 10 of 16 patients with a positive diazo reaction, at the time of writing are dead, and the others are all in a critical condition with a poor prognostic outlook. The majority of our cases had the positive reaction only over a relatively short period of time, but rare cases of chronic glomerulonephritis have been reported where the positive diazo reaction has been noted over a long period of months.^{18, 19}

In our experiments spectrophotometric analysis demonstrated that the chromogen of the diazo reaction on plasma of patients with a high plasma indican was identical with the curves obtained on pure solutions of indican made up to those concentrations which exist in the blood of uremic patients. This seems to be conclusive proof that the diazo reaction is dependent on a high plasma indican. Further, it has been demonstrated that this level must be 15 mg per cent or higher before a positive reaction is noted.

If these conclusions are correct the diazo reaction, by reason of its simplicity, becomes the practical clinical test of choice for it both indicates that the plasma indican is elevated to at least three times the normal range and that a fatal termination may be expected in a relatively short period of time.

It has not been possible to demonstrate a definite correlation between an increase of the plasma indican and the symptomatology of uremia, and it is felt that indican is probably not a toxic substance. This statement seems justified since Earlam and Bolliger⁹ have shown that the presence of indican in the plasma of dogs suffering from renal insufficiency, in amounts several times greater than the highest yet recorded in fatal (uremic) cases, caused no untoward effects whatever.

CONCLUSIONS

A reliable method for indican determinations has been adapted to a photoelectric colorimeter, thus obviating the constant use of indican as a standard and removing the inherent errors of ordinary color comparison colorimetry.

Indican, a normal constituent of blood, is elevated in cases with renal insufficiency which also exhibit a retention of nitrogenous products and an impaired phenolsulphonphthalein excretion.

The diazo reaction is due to the presence of indican and becomes positive when the level of the latter reaches 15 mg per cent and is a more useful practical test than indican assay in renal insufficiency because of the ease of determination and the prognostic import that it conveys.

There appears to be no definite correlation between the symptomatology of uremia and the raised plasma indican.

The author wishes to acknowledge a debt of gratitude to Dr J P O'Hare for his advice and criticism in the preparation of this paper.

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TRANSIENT ELECTROCARDIOGRAPHIC CHANGES NOTED DURING ATTACKS OF ANGINA PECTORIS WITH REPORT OF A CASE*

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THE opportunity of obtaining an electrocardiogram during the acute phase of an anginal seizure does not often present itself. For obvious reasons, one hesitates to induce an attack of angina pectoris simply for the purpose of securing such a record and consequently the literature contains relatively few reports dealing with such phenomena, particularly cases in which syphilitic aortitis and coronary thrombosis can be definitely ruled out. The changes in the electrocardiogram, although transient, are so striking and in general so consistent as to be of considerable clinical interest. The cases which have been reported to the present time deal almost entirely with alterations in the standard limb leads, and so far as we have been able to find none include a study of the chest lead. In the case presented here all four leads were obtained before, during, and after the anginal seizure thus permitting a study of the changes occurring in the chest lead as well as in the three standard leads.

The changes which have been commonly reported^{1,2} in uncomplicated angina pectoris tracings consist of deviation of the RS-T segment and alteration in the amplitude and direction of the T waves. In the observations of Feil and Siegel² the most outstanding finding was a definite depression of the RS-T segment in Leads I and III with a prompt return to its previous status after cessation of the pain. Parkinson and Bedford,¹ in the study of five cases, noted depression of the RS-T segment and decreased amplitude or inversion of the T waves in one or two leads followed by almost complete disappearance of these changes after the seizure had passed off. Brown and Holman⁴ find that the most important electrocardiographic changes consisted of "alteration in the terminal deflections."

CASE HISTORY

C. M., a white male, aged 47 years, was admitted to the Wisconsin General Hospital on November 2, 1936 complaining of "pain beneath the breast bone." He dated the onset of this condition to five years ago when he was suddenly seized with an attack of substernal pain, epigastric discomfort, and nausea coming on about one half hour after shoveling snow. Belching of gas a short while later relieved his symptoms entirely. Similar episodes occurred intermittently until three months previous to admission, since which time the symptoms had become more severe and more frequent, coming on after the slightest exertion, usually accompanied by vomiting, dysphagia, palpitation, and dyspnea. Laryngoscopy, chest, gall bladder, and stomach x-ray studies repeated at intervals had been negative. The past medical history

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revealed the occurrence of measles, tonsillitis, mumps, smallpox, influenza, whooping cough, pneumonia, and pleurisy in early life. The family history was interesting in that his mother died at the age of 58, with a condition similar to that of the patient.

To physical examination the patient showed slight tortuosity of the retinal vessels and moderate peripheral sclerosis. The pulse was regular at a rate of 72. The blood pressure was 122/70. There was no cardiac enlargement on percussion. The heart sounds were of fairly good quality at all areas and no murmurs were heard. The lungs and abdomen were negative, and there was no edema of the extremities.

The results of the routine laboratory studies were within normal limits. X-ray examination of the esophagus and gastrointestinal tract revealed no abnormalities. Orthodiascopic studies showed the heart to be within normal limits as to size and shape.

On November 9 the patient was sent to the cardiology department for an electrocardiogram. The exertion incident to the trip to the laboratory precipitated an anginal attack during which the electrocardiogram, a portion of which is reproduced in Fig. 1, was recorded.

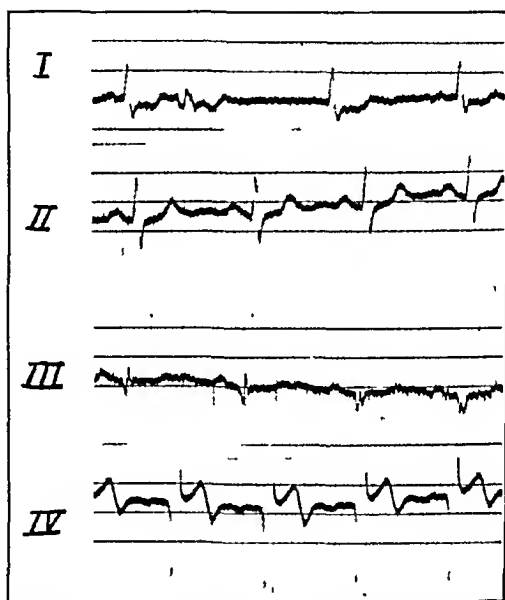


Fig. 1.—Electrocardiogram taken on Nov. 9, 1936, during the initial anginal attack precipitated by walking to the electrocardiographic laboratory. Note, especially, the diphasic T₁.

The complete record showed two ventricular extrasystoles in Lead I, followed by sinoauricular block with auriculoventricular nodal escape. The dominant rhythm was a sinus arrhythmia at a rate of 75 to 90. There was a large Q₂, depression of the RS-T segments in Leads I and II, elevation of the RS-T segment in Lead IV, and a diphasic T₁ and T₄.

From the standpoint of scientific interest it was felt desirable to obtain a series of tracings before, during, and after such an attack, in an attempt to determine whether these changes could be consistently reproduced. With the permission and cooperation of the patient such a series was obtained two days later. The initial control electrocardiogram taken with the patient symptomless is shown in Fig. 2A. It will be noted that this tracing exhibited a deep Q₂, slight depression of the RS-T segment in Lead I, and slight elevation of the RS-T segment in Lead III. T₁ and T₂ were almost flat, and T₄ was normally inverted. These changes will be recognized as characteristic of coronary sclerosis. The patient was then asked to walk up a short flight of stairs, an exercise which he had found almost invariably precipitated attacks of substernal pain. During the seizure which followed, the tracing shown in Fig. 2B was recorded. On this occasion there was a marked sinus arrhythmia with a rate

varying from 45 to 90. The RST segment became more definitely depressed in Lead I and elevated in Lead IV. T_1 and T_2 became diphasic and somewhat increased in amplitude, T_3 deeply inverted, and T_4 diphasic but chiefly upright. Five minutes later the symptoms had completely disappeared and the electrocardiogram taken at this time, shown in Fig 2C, revealed almost complete return to the preanginal status, with the exception of very slight inversion of T_1 and a slight increase in the amplitude of T_3 and T_4 .

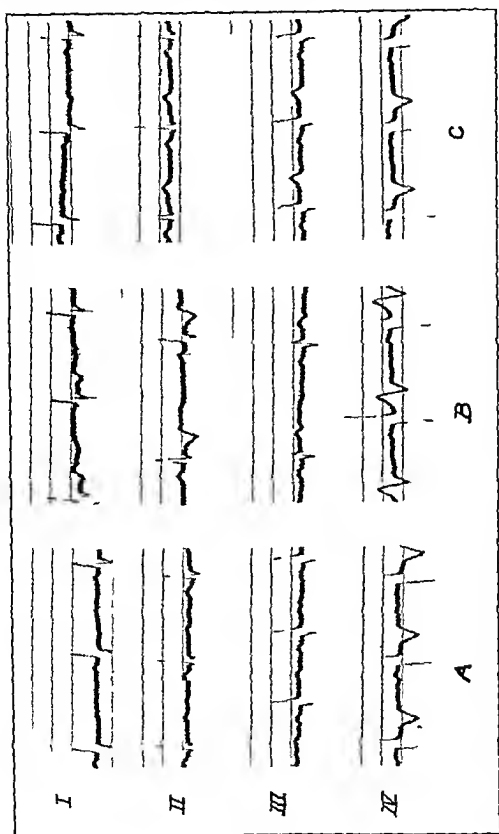


Fig 2.—Electrocardiogram taken on Nov 11, 1936. A Before anginal attack. Note deep Q 3. Practically isoelectric RST segments. Almost flat T_1 and T_3 . T_4 normally inverted. B Taken on a few minutes later during anginal attack. RS T 1 more definitely depressed. RS T 3 more definitely elevated. T_1 , T_2 , and T_4 diphasic. T_3 diphasic but chiefly upright. C Taken five minutes later after symptoms had entirely disappeared. Tracing has returned very nearly to preanginal status. T_4 has resumed its inverted position.

DISCUSSION

The changes seen in the electrocardiogram during a seizure of typical angina pectoris are recognized as being practically identical with those encountered following acute coronary occlusion, differing only in the point of

duration. In the case presented this was further borne out by the changes in the chest lead characterized by the deviation of the RS-T segment and a tendency for the T-wave to turn upright. This lends further support to the now generally accepted view that the symptoms of coronary insufficiency are due primarily to myocardial ischemia of a transient nature in angina pectoris and of considerable duration following coronary occlusion.

SUMMARY

A case is presented in which electrocardiograms were taken before, during, and immediately following an attack of typical angina pectoris. The chest lead was taken in addition to the three standard limb leads, and the changes in the tracing taken during the attack were characteristic of acute coronary occlusion with the exception of their transitory nature.

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THE PARTITION OF NITROGEN IN CANINE GASTRIC JUICE

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THE partition of nitrogen in the gastric juice has been the subject of much controversy. Rosemann (1927) and Carlson (1915, 1919) maintain that practically all the organic solids of the gastric juice are represented by proteins (Carlson) or "a protein-like body" (Rosemann). This view was shared by Dienst as recently as 1931. On the other hand, it has been asserted that the gastric juice, or rather the gastric contents, in normal persons and in various clinical cases contain considerable amounts of nonprotein nitrogen, ranging from 16 to 200 mg. per cent (Polland, Roberts, and Bloomfield, 1926; Martin, 1931; Mielke, 1932; Baltzer, 1934; Burger, Hartfall, and Witts, 1933; Diehl, 1936). The amount of nonprotein nitrogen in the gastric juice was found to be greatly increased in cases of achylia gastrica, or carcinoma, and particularly of nephritis accompanied by retention of nitrogen. Ammonia was

early recognized to be a normal constituent of gastric juice, forming a considerable part of the total nitrogen content of the secretion (Nencki, Pavlov, and Zaleski, 1898). This finding was later confirmed by many other investigators (Salaskin, 1898, 1902; Carlson, 1915; Rosemann, 1927; Martin, etc.). It has been shown that the gastric juice contains a small quantity of urea (Martin, 1931; Komarov, 1931). Mielke regards all the nonprotein nitrogen of the gastric juice as urea, although his opinion was not substantiated by any experimental evidence. The presence of creatine bodies, uric acid, adenosin phosphoric acid, and amino nitrogen has been postulated by many investigators (Toni and Marciali, 1928; L. Martin; Baltzer; Diehl), but none of the substances belonging to this group was isolated from the gastric juice and identified chemically.

It should be mentioned that only one investigation—that of Rosemann (1927)—has been carried out on a large scale and with really pure gastric juice (obtained by sham-feeding from a dog with a gastric fistula and esophagotomy). Rosemann found that the organic matter in gastric juice consists practically entirely of a proteinlike body, the remainder being so minute in amount that it is not even worthy of mention. It should be noted that in all the studies in which large percentages of nonprotein nitrogen have been reported, the methods of routine blood analysis had been applied to the human gastric contents, the samples having been obtained from fasting subjects, sometimes without stimulation but more often after the injection of histamine, under normal or pathologic conditions. The wide divergences in the analytical results obtained even in the course of one and the same investigation (cf., for example, the work of Martin and of Mielke) are in all probability largely due to contamination with saliva, bile, or intestinal juice, all of which are known to contain large amounts of nonprotein nitrogen (for references, see Babkin, 1928). In the opinion of the writer the discrepancies might also be attributable to the inadequacy of the methods used. The proteins of the gastric juice are entirely different from the proteins of the blood (Webster and Komarov, 1932) and, therefore, it is questionable whether the methods used in blood analysis are suitable for the determination of nonprotein nitrogen in the gastric juice.

The main purpose in the present investigation was to study the efficacy of some of the common protein precipitants when applied to the proteins of the gastric juice, with the object of developing a convenient and yet reliable method for routine determinations of nonprotein nitrogen in gastric secretion. Among the methods tested, particular attention has been paid to the standard methods employed in blood analysis.

The partition of nitrogen in gastric juice was also studied. It was recently shown by the writer that pure canine gastric juice contains a number of physiologically active substances belonging to the group of nitrogenous bases (vitamin B, histamine, and guanidine-like bodies). Fractionation of these substances was effected mostly by the Kossel and Kutscher method, somewhat modified (Komarov, 1934, 1936). For this reason in our analysis

special emphasis has been placed on the nitrogenous bases of gastric juice and the distribution of nitrogen among the various fractions resulting when the method of Kossel and Kutscher is applied to the gastric juice.

METHODS

Gastric Juice.—The gastric juice employed in this study was obtained, in response to sham-feeding or subcutaneous injection of histamine dichloride (0.05 mg. per kg. body weight), from dogs with a gastric fistula and esophagotomy. Whenever there was the slightest suspicion that the secretion had been contaminated with food residues, blood or intestinal juices, the presence of the last-mentioned being indicated by the appearance of bile, the samples were discarded. Samples of clear gastric juice were collected at fifteen-minute intervals, immediately filtered and analyzed. In order that the effect of autodigestion might be observed, some samples of juice were allowed to stand for varying periods of time at room temperature or in the incubator at 38° C.

Protein-Free Filtrates From Gastric Juice.—The efficacy of the following procedures in precipitating the proteins of gastric juice was studied.

1. *Precipitation With Acetone.*—To the acid gastric juice (acidity about 150 m.eq. per liter), two volumes of acetone were added. Usually this was carried out in 15 c.c. centrifuge tubes, containing 10 c.c. of acetone, into which 5 c.c. of gastric juice was poured, the two liquids being thoroughly mixed. The tubes were corked and allowed to stand overnight. After the mixture had been centrifuged, the supernatant liquid was decanted and rapidly filtered through cotton wool. Allowance was made for the change in volume due to the volatility of acetone and the contraction which takes place when acetone is mixed with water. Sometimes it was found more convenient to filter the supernatant liquid into a 25 c.c. measuring flask, and to wash the precipitate on the centrifuge twice with 5 c.c. of 66 per cent acetone saturated with NaCl. The two modifications gave identical results.

2. *Coagulation by Heat.*—Five or 10 c.c. of gastric juice was placed in a 15 c.c. graduated centrifuge tube, loosely corked with a rubber stopper, and the tube was then immersed in a boiling water-bath. Complete coagulation usually occurred within two minutes, after which the tubes were immediately cooled under running water. The precipitate was removed either by centrifuging or by filtering. In order to ensure complete coagulation, the acidity of the gastric juice required to be about 150 m.eq. per liter.

3. *Precipitation With Trichloroacetic Acid.*—Several procedures involving the use of trichloroacetic acid have been studied.

(a) Five cubic centimeters of gastric juice were mixed with 5 c.c. of 15 per cent trichloroacetic acid in a 15 c.c. graduated centrifuge tube. The tube, loosely corked with a rubber stopper, was immersed in a water-bath at 80° C. for five minutes, and then immediately cooled under running water. The precipitate was separated by centrifuging, and the supernatant fluid filtered. This procedure was soon found to be one of the most reliable.

The following procedures based on the use of trichloroacetic acid were also studied, although they were soon found to be unreliable

(b) Five cubic centimeters of 15 per cent trichloroacetic acid were mixed with 5 cc of gastric juice and allowed to stand overnight at room temperature. In all cases only a very faint opalescence could be observed.

(c) The gastric juice was first neutralized with 0.2 N NaOH, the necessary amount of the latter having been determined previously by titration against methyl red. To 5 cc of neutralized gastric juice, 5 cc of 15 per cent trichloroacetic acid was added, and the mixture allowed to stand overnight. By this method some protein was always precipitated, but the precipitation was never complete.

(d) The gastric juice was neutralized with NaOH mixed with trichloroacetic acid as in procedure (c), and the mixture was finally subjected to heating at 80° C for five minutes. More protein was precipitated than by procedure (c), but the precipitation was never complete.

4 *A Modification of Somogyi's Procedure*—Five or 10 cc of gastric juice was placed in a 25 cc measuring flask, 1 cc of 10 per cent $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 2 drops of 0.5 per cent phenolphthalein were then added. 0.5 N NaOH was next added, until the mixture became slightly pink after which 0.1 N HCl was added drop by drop (from 1 to 3 drops usually being required), until the supernatant fluid became colorless while the precipitate still remained slightly pinkish. The volume was made up by the addition of distilled water. Filtration was carried out immediately or the next day.

5 *Tungstic Acid Precipitation*—In the present study all attempts to utilize tungstic acid for quantitative precipitation of the proteins of the gastric juice were unsuccessful.

(a) *Precipitation at pH 6*—In accordance with the principles of the original Folm Wu method this was carried out as follows. 5 or 10 cc of gastric juice were neutralized with 0.2 N NaOH, 1 cc of 10 per cent sodium tungstate, and the amount of $\frac{2}{3}$ N H_2SO_4 necessary to adjust the reaction to pH 6.0, were added. In no case was any precipitation observed when pure, freshly secreted gastric juice was treated in this way.

(b) *Precipitation at pH 3.5*—This was carried out exactly as suggested by Martin (1931).

(c) *Precipitation at pH < 1.0*—It was observed that an increasing amount of nitrogen was precipitated from gastric juice by means of tungstic acid as the acidity of the medium was increased. The nitrogen content of the filtrates was lowest when the following procedure was employed. To 5 cc of gastric juice 1 cc of 10 per cent sodium tungstate and 1 cc of 1 N sulfuric acid were added. No precipitation immediately occurred, some precipitation took place after a few hours. The mixture was allowed to stand for several days at room temperature until no more turbidity appeared overnight in the supernatant fluid after centrifugalization. The filtrate was obtained by centrifuging at high speed and then filtering.

TABLE I
NONPROTEIN NITROGEN IN GASTRIC JUICE AS DETERMINED BY THE VARIOUS METHODS STUDIED

GASTRIC JUICE			NITROGEN IN THE FILTRATES (MG. %)										5 SO- MOGYI'S METHOD	PROTEIN NITRO- GEN (MG. %)
SAM- PLS	STIMULATION	STATE OF PRESERVATION	TOTAL NITROGEN (MG. %)	1 ACETONE	2 HEAT COAGULA- TION	3A T.C.A. DIRECT 80°	3C		3D NEUTRALIZATION T.C.A.	4B	TUNGSTIC ACID			
							20°	80°			AT pH 3.5	AT pH < 1.0		
1	Sham-feeding	Fresh	27.20	12.04	14.56	13.44	-	-	-	No ppt.	-	6.08	9.80	13.16
2	Sham-feeding	Fresh	21.30	8.10	8.10	8.10	-	-	-	No ppt.	-	-	7.60	13.20
3a	Sham-feeding	Fresh	19.20	7.02	7.42	7.38	12.40	10.70	-	No ppt.	-	4.12	5.63	13.18
3b	(Same sample)	24 hr. at 20° C.	do.	7.42	8.12	-	-	-	-	No ppt.	-	5.49	5.74	11.78
3c	(Same sample)	24 hr. at 38° C.	do.	14.00	14.00	14.00	-	-	-	No ppt.	-	8.63	11.20	5.20
4a	Psychic	Fresh	16.50	6.58	-	-	-	-	-	13.40	-	-	6.58	9.92
4b	(Same sample)	24 hr. at 38° C.	do.	13.00	13.60	13.45	13.60	-	-	No ppt.	-	-	9.94	3.50
5	Histamine: total secretion	Fresh	8.82	5.88	6.90	6.72	-	-	-	-	-	-	4.48	2.94
6	Histamine: total secretion	Fresh	8.80	6.72	7.30	7.14	-	-	-	No ppt.	-	3.79	4.76	2.08
7	Histamine: total secretion	Fresh	12.20	9.52	9.84	9.66	-	-	-	No ppt.	-	4.57	7.85	2.68
8	Histamine: secretion for first ¼ hr.	Fresh	16.80	8.96	9.80	9.66	-	-	-	-	-	-	7.84	7.84
9	Histamine (same expt. as 8): secretion for second and third ¼ hours.	Fresh	13.16	5.88	7.56	7.28	-	-	-	No ppt.	-	3.83*	4.90	7.28
10	Secretion from pouch (after 2 weeks at 20° a meal) — contaminated with exudate from ulcerated skin	2 weeks at 20° to 30° C.	92.00	78.40	83.20	87.40	87.80	-	-	56.00	-	48.20	56.70	?

*Nitrogen in the filtrate after precipitation with phosphotungstic acid in the presence of 5 per cent sulfuric acid amounted to 3.13 mg. per cent.

Partition of Nitrogen in Gastric Juice—Some data (not hitherto reported) on the partition of nitrogen in gastric juice were obtained in the course of previous studies, during which several large samples of gastric juice—amounting altogether to more than 100 liters—were fractionated by different methods (Komarov, 1933, 1934, 1935, 1936). On the basis of these findings it is believed that the addition of two volumes of acetone to freshly secreted gastric juice brings about a complete precipitation of the protein material. The distribution of nitrogen, both in the precipitates and the filtrates obtained by the use of acetone, was studied on several occasions and found to be remarkably consistent. The chemical properties and the composition of the protein precipitate have been described previously; the protein was found to be a mucoprotein having much in common with the pepsin preparations described by Pekelharing (Webster and Komarov, 1932, Komarov, 1935). The present paper affords an opportunity of describing our data concerning the partition of nitrogen in the acetone filtrates, thus making the report of the analysis of several large samples of gastric juice complete. Fractionation was carried out by the method of Kossel and Kutscher, somewhat modified. The fraction of the nitrogenous bases was isolated by precipitation with phosphotungstic acid, and the pumme, histidine, arginine, and lysine fractions by the silver baryta method. Details of the procedure used have been given in the articles in which the physiologic properties of these fractions were reported (Komarov, 1933, 1934, 1936). In addition volatile bases, urea and creatine bodies were determined in the protein free filtrate before fractionation.

Nitrogen was determined by micromodifications of the Kjeldahl method (Pregl, 1930). Urea and ammonia plus volatile bases were determined by the mase aeration titration method of Van Slyke and Cullen, creatine plus creatinine by Fohn's open flask method (see Peters and Van Slyke, 1932).

EXPERIMENTAL RESULTS AND DISCUSSION

The analytical data obtained are presented in Tables I and II. Table I shows the amount of nitrogen present in the filtrates obtained by the methods outlined above. Table II shows the partition of nitrogen in the filtrates obtained by the acetone method from large samples of freshly secreted, pure gastric juice obtained in response to sham feeding.

Of the various methods for obtaining protein free filtrates from gastric juice that were studied in the present investigation, only the following three gave consistent and comparable results:

- (1) precipitation with acetone,
- (2) heat coagulation in an acid medium, and
- (3) precipitation with 75 per cent trichloroacetic acid at 80° C.

When absolutely fresh canine gastric juice was precipitated with two volumes of acetone, filtrates were obtained which, after the removal of the acetone, showed a negative biuret reaction. It was also found that the precipitates obtained from different samples of gastric juice have a consistent

elementary composition (Webster and Komarov, 1932; Komarov, 1935). On this account precipitation with acetone was selected as the basis for comparison of the efficacy of the other methods.

The average figures obtained by the various methods were computed from the data presented in Table I. Direct boiling (method 2) gives figures for

TABLE II

PARTITION OF NITROGEN IN GASTRIC JUICE OBTAINED IN RESPONSE TO SHAM-FEEDING

NITROGEN IN THE FORM OF:	SAMPLE A (4 LITERS) MG. %	SAMPLE B (6 LITERS) MG. %
Protein	18.00	19.90
Nonprotein	9.80	10.90
Urea	0.16	0.11
Creatine + Creatinine	0.09	0.11
Bases, total	5.37	6.59
Bases, volatile	1.78	2.55
Bases, nonvolatile	3.59	4.01
Bases, purine fraction	0.11	0.10
Bases, histidine-arginine fraction	1.56	1.77
Bases, lysine fraction	1.88	2.17
Monoaminoacid fraction (phosphotungstic acid filtrate)	0.70	1.02
Humine bodies	3.73	3.29

nonprotein nitrogen that are 13.5 per cent higher than those obtained by precipitation with acetone. In most cases the filtrates showed a very slight, but nevertheless distinct, positive biuret reaction, the color developed being violet blue. Precipitation with trichloroacetic acid at 80° C. gives figures for non-protein nitrogen 10 per cent higher than those given by acetone precipitation. If absolutely fresh gastric juice, obtained by means of sham-feeding, was used, the filtrates always showed a negative biuret reaction. If, however, the juice was treated with trichloroacetic acid after standing for a few hours at room temperature, a slight positive (violet) biuret reaction was obtained. In "histamine" gastric juice the trichloroacetic acid filtrates always showed a positive biuret reaction, the color developed being pure blue, if the juice was precipitated at once, or displaying a slightly violet tinge, if the juice was not quite fresh. In connection with these results, it should be mentioned that Pekelharing as long ago as 1902 observed some extremely interesting facts regarding heat coagulation of pepsin. He found that pepsin can be precipitated by heating only when the pepsin solution is acid and is heated to the point of coagulation very rapidly. This rule holds true for solutions of either crude or highly purified preparations of pepsin and also for gastric juice. Pekelharing also pointed out that under such conditions pepsin is partially hydrolyzed to form a product which gives a precipitate, and which he termed "*Gerrinnungsprodukt*," and a certain albumose-like body which remains in solution. If the solution of pepsin is heated slowly, the hydrolysis is carried much further and less precipitate is formed. Under sufficiently slow heating, no precipitation occurs whatsoever. In our experiments the method of direct heat coagulation yielded filtrates showing a positive biuret reaction, and having higher contents of nitrogen than those obtained with the trichloroacetic

acid procedure. These results can be satisfactorily explained by the assumption that Pekelhaug's albumose is precipitated by trichloroacetic acid.

Special experiments were carried out with a view to studying the influence of acidity on the completeness of heat coagulation. The results of a typical experiment of this kind are given in Table III.

TABLE III

REACTION OF GASTRIC JUICE AND PRECIPITABILITY OF THE PROTEIN MATERIAL BY HEAT

SAMPLES	GASTRIC JUICE CC	0.2N NaOH CC	CO ₂ FREE H ₂ O CC	pH	NITROGEN IN THE FILTRATES MG % OF GASTRIC JUICE	NITROGEN PRECIPITATED MG % OF GASTRIC JUICE
1	5.00	0.00	5.00	-	16.23	13.17
2	5.00	2.00	3.00	1.80	21.00	8.40
3	5.00	2.50	2.50	2.00	22.40	7.00
4	5.00	3.00	2.00	2.70	23.50	5.90
5	5.00	3.50	1.50	3.00	25.20	4.20
6	5.00	3.60	1.40	5.40	24.40	5.00
7	5.00	3.65	1.35	5.80	25.80	3.60

The gastric juice employed in this experiment was obtained from a dog with a gastric fistula and esophagotomy in response to continuous sham feeding with raw minced meat. The secretion collected during a forty minute period was immediately filtered and used for the above experiment. The total acidity and the free acidity were respectively 168 and 144 meq per liter. The total nitrogen was 29.40 mg per cent. The nonprotein nitrogen as determined by the acetone method and by precipitation with 7.5 per cent trichloroacetic acid at 80° C was found to be 16.2 mg per cent. The samples treated as indicated in the table were heated simultaneously in a boiling water bath for five minutes, cooled simultaneously in a cold water bath and filtered through Whatman's filter paper No. 42. pH values were only roughly determined by means of B. D. H. capillator colorimeter.

It is evident that, with the progressive lowering of the acidity of the gastric juice by the preliminary addition of sodium hydroxide, less and less protein material is coagulated by heating until at a pH above 5.8 no precipitation takes place whatsoever.

In the filtrates obtained by the addition of trichloroacetic acid to fresh gastric juice, secreted in response to sham feeding, and subsequent heating to 80°, the biuret reaction is negative, but with "histamine" gastric juice the filtrates always show a blue color. In this connection it should be mentioned that the main bulk of protein material in the gastric secretion elicited by sham feeding consists of the protein described by Webster and Komarov (1932) as a mucoprotein of the gastric secretion. This mucoprotein has a nitrogen content of about 14.0 per cent. It was found recently by the writer (unpublished data) that the main bulk of the protein of the "histamine" gastric secretion consists of an entirely different protein, containing a lower percentage (i.e. 12 per cent) of nitrogen. This protein has also the more pronounced characteristics of a mucin since after preliminary acid hydrolysis it shows a very high reducing power equivalent to a 30 to 33 per cent glucose content. Apparently the former protein is precipitated completely by trichloroacetic acid, whereas for the latter precipitation is not complete. This problem is not yet entirely solved; further study of it is now in progress.

When trichloroacetic acid is added directly to gastric juice of normal acidity (about 0.15 normal) in an amount of from 5 to 10 per cent, no pre-

precipitation takes place within twenty-four hours, if the mixture is kept at a temperature not exceeding 20° C. However, at temperatures above 24° C. precipitation of protein occurs, and at 38° C. the proteins of the gastric juice are completely precipitated by trichloroacetic acid within two or three hours. Special experiments were performed which showed that under such conditions gastric juice in the presence of 7.5 per cent trichloroacetic acid exhibits considerable digestive power. These observations strongly suggest that the protein material present in freshly secreted gastric juice is not precipitable by means of trichloroacetic acid at room temperature, but that the proteins of the gastric juice acquire this property at a certain stage of hydrolysis. Probably that is also the explanation of another fact observed in the course of this study, namely, that, when trichloroacetic acid is added to previously neutralized gastric juice, it always causes some precipitation of protein material at 20° C.; the precipitation, however, is never complete, even when supplemented by heating to 80° C.

The modified form of Somogyi's method which we have employed likewise yielded very consistent results. With freshly secreted gastric juice, the values for nonprotein nitrogen were about 10 per cent lower, and with autolyzed gastric juice they were about 20 per cent lower than those obtained by the acetone method.

The Folin-Wu method, in the various modifications used, was found to be entirely unreliable. At pH higher than 3.5, tungstic acid does not produce any precipitation. With the increase of the acidity more and more nitrogen is precipitated until, at pH less than 1.0, the amount of nitrogen precipitated very closely approximates the amount precipitated by phosphotungstic acid.

Table I (samples 3a, 3b, 3c, 4a, and 4b) shows that the most rigid precautions should be taken to prevent autodigestion of the gastric juice, if it is desired to obtain true values for nonprotein nitrogen, since a considerable increase in nonprotein nitrogen occurs within twenty-four hours even at room temperature, whereas at 38° C. from 66 to 75 per cent of the protein originally present is hydrolyzed.

The analysis of sample 10 demonstrated that even a very slight, almost imperceptible, contamination of the gastric juice by an exudate from the ulcerated skin around the fistula may lead to an entirely wrong conception regarding the composition of the gastric juice. It is evident that the absolute figures for the various fractions of nitrogen in sample 10 do not admit of comparison with the corresponding figures for really pure gastric juice. In contrast to the results obtained with pure gastric juice, tungstic acid caused considerable precipitation at pH 6 or pH 3.5. This result was apparently due to the presence of blood proteins in various stages of hydrolysis.

From the experimental data presented in this article certain conclusions may be drawn with regard to the composition of gastric juice, as affected by the nature of the stimulus producing it. The gastric secretion elicited through the vagal mechanism (sham-feeding juice and psychic secretion) differs strikingly in composition from "histamine" juice, especially in regard to its

content of protein nitrogen The greatest concentration of protein nitrogen was observed in the sham feeding juice, which represents the glandular response to nervous stimulation in its most intense form The protein nitrogen content is considerably lower in the psychic secretion, and still lower in the typical histamine secretion (samples 5, 6, and 7) When psychic impulses are not entirely excluded, the protein nitrogen content of the histamine juice approximates that of the psychic secretion (samples 8 and 9) Nonprotein nitrogen forms a considerable part of the total nitrogen of gastric juice under all conditions of stimulation In the vagus juice it constitutes from 36.5 to 44 per cent of the total nitrogen, in the typical histamine juice the proportion is much higher, viz, from 67 to 78 per cent The absolute figures for nonprotein nitrogen vary from 12.04 mg per cent in the sham feeding juice to 6.02 mg per cent in the psychic secretion, and from 9.52 to 5.88 mg per cent in the histamine juice

From the experience gained in this laboratory over a number of years, it appears that the variations both in protein and nonprotein nitrogen shown in Table I cover fairly well the range of variations which may be observed in the secretion of the whole stomach in normal dogs under experimental conditions The values for nonprotein nitrogen in the gastric contents of human beings, as reported in the literature, are in most cases much higher Pollard, Roberts, and Bloomfield reported 40 to 80 mg per cent of nonprotein nitrogen, Martin 20 to 48 mg per cent, Mielke 28.4 to 85.5 mg per cent, and Diehl 27.1 to 86.8 mg per cent It should be noted that these data were obtained by the Folin Wu method in its original, or in a somewhat modified form, or by trichloroacetic acid precipitation at room temperature On the other hand, our figures for nonprotein nitrogen are fairly close to those reported by Baltzer (1934), which were obtained by the ultrafiltration method, or by means of precipitation with ferric hydroxol

The partition of nonprotein nitrogen was studied in the acetone filtrates obtained from two large samples (4 and 6 liters, respectively) of gastric juice secreted in response to sham feeding, as shown in Table II Urea, ammonia, and other volatile bases, and creatine constitute about 20 per cent of the nonprotein nitrogen About 37 per cent of the nonprotein nitrogen is represented by the physiologically important fraction of nitrogenous bases precipitable by phosphotungstic acid (volatile bases excluded) The amount of nitrogen precipitated by silver nitrate at pH 2.8 and pH 7.0—"purine" and "histidine" fractions—is quite negligible Almost the whole bulk of the nitrogenous bases is present in the "arginine" fraction precipitable by silver nitrate and barium hydroxide at pH 8.0 to 9.0 and in the filtrate of the former—"lysine" fraction This observation is of some interest, in view of the previously described experiments, which showed that the arginine fraction exerts a histamine like action on blood pressure and also on gastric, pancreatic and biliary secretion, and that the lysine fraction gives a positive Sakaguchi reaction and shows a guanidine like action on skeletal muscles, blood pressure, and on pancreatic and biliary secretion (Komarov, 1935)

SUMMARY

1. Three methods for the determination of nonprotein nitrogen in gastric juice were found to give uniform and comparable results: heat coagulation, precipitation with two volumes of acetone, and precipitation with trichloroacetic acid at 80° C. The last two methods were found to be particularly useful for routine work.

2. In regard to the partition of nitrogen there was a striking difference between the gastric secretion elicited by the vagal mechanism (sham-feeding) and that provoked by histamine.

3. A more detailed study was made of the partition of nitrogen in gastric juice secreted in response to sham-feeding, which among other data revealed the fact that a surprisingly large proportion of nonprotein nitrogen (about 40 per cent) is formed by the physiologically important fraction of nitrogenous bases.

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STILL'S DISEASE WITH HYPERGLOBULINEMIA

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IN 1897, Still¹ described a form of arthritis in children which has since then been called by his name. The syndrome was characterized by a mild non-destructive arthritis, a swelling of the lymph glands, enlargement of the spleen and fever. The arthritis was multiple and usually recurrent. The lymphadenopathy was general, the glands, while firm, never exceeded the size of a nut. The splenomegaly, sometimes minimal, was occasionally marked, the spleen in these cases extending below the level of the navel. The fever was never high and was sometimes continuous, sometimes recurrent, it bore no relation to the severity of the arthritis. Of his 12 cases 3 came to autopsy, all three showed an adhesive pericarditis that had not been diagnosed before death.

A year earlier Chauffard and Ramond had described 7 cases of a similar syndrome but without the splenomegaly. Moreover, their patients were all adults, ranging in age from 22 to 61 years. Since then, especially in France, the condition is often called the *Chauffard-Still syndrome*.

During the past thirty years a considerable number of cases of Still's disease have been described, chiefly in England and Germany, more recently in this country and in France. A good analysis of the cases reported up to 1930 may be found in the article of Chevalier.² The disease may occur at any age, but is most characteristically a disease of the young, one third of the cases have been found in childhood, one third during adolescence, and one third beyond the age of 25 years, the oldest reported being 61 years old.^{3, 4} While a nondestructive periarthritis is an essential feature of the disease, this symptom may vary from an extreme form producing complete crippling to a relatively mild involvement with intermissions. The adenopathy, while uniformly present, varies greatly in extent, the glands are never very large and in general the degree of adenopathy varies with the intensity of the joint involvement. The splenomegaly also varies, being occasionally absent. Usually the spleen can be felt a few fingerbreadths below the costal margin and is sometimes enormous, extending below the navel. Fever is rarely absent and while sometimes inconsiderable, may run a high septic course for weeks at a time. There is always progressive emaciation and a marked secondary anemia. The white count usually shows a moderate leucocytosis early in the disease. Later a leucopenia, sometimes extreme,⁵ is the rule. Of the complications pericarditis is the most frequent. It is usually fibrous, leading to obliteration of the pericardial sac, but may be exudative. Pleuritic adhesions are constant.

The autopsy findings usually show no very characteristic changes. The joints show periarthritic thickening, the synovial membrane being usually not

involved. The lymph glands show only indurative processes in the interior, no specific changes.⁶ The splenic changes are limited to a hyperplasia of the cellular elements and a moderate congestion; there is no fibrosis.⁴

It is obvious that Still's disease, as extended by later observers, is not a sharply defined symptom complex. If applied to adults it certainly includes cases of Felty's syndrome⁷ which both clinically and pathologically are indistinguishable from Still's disease. Cases in which the arthritis is minimal but in which fever, leucopenia, and splenomegaly are marked would suggest Banti's disease; if the peritoneum were involved, as the pericardium and pleura so often are, the two diseases would be nearly indistinguishable. However, I do not know of any case of Still's disease with ascites, and the liver is enlarged only if there is a passive congestion due to heart failure or if the organ is the site of amyloid degeneration.⁸ Moreover, since moderate adenopathy and slight splenic enlargement are not uncommon in children suffering from any infection, it is difficult to draw the line between ordinary cases of juvenile polyarthritis and some cases of Still's disease. Smith and Gallup⁹ go so far as to say: "This condition was formerly spoken of as 'Still's Disease,' but the name has now been generally discarded and the condition is recognized merely as a modification of atrophic arthritis."

Whatever one may conclude regarding the justification of considering the Still-Chauffard syndrome as a clinical entity, it will remain a useful means for gathering together that group of cases, no longer rare, that show a peri-arthritis, splenomegaly, adenopathy, fever, anemia, and usually involvement of pericardium and pleura and in which all the more usual diagnoses can pretty definitely be excluded. It is for this reason that the two cases reported here, certainly not typical of the disease as described by Still, have been included within this group.

Within the last few years increasing attention has been directed to the visceral manifestations of lupus erythematosus disseminatus. These consist of a long-continued fever, arthritis, pleurisy, pericarditis, pneumonitis, nephritis, and leucopenia. Disseminated cerebral manifestations may occur and in all of our cases that were observed for a sufficient length of time, high blood serum globulin and englobulin values, quite comparable to those reported below, were found. The suspicion is justified that cases of disseminated lupus erythematosus without skin manifestations may not be uncommon, and that lupus, the Libman-Sacks endocarditis, the Still-Chauffard syndrome, and Felty's disease may all be manifestations of the same disorder. The final solution of this question must await the discovery of a common etiologic agent, if any.

The technique of blood protein determinations used at the laboratory of the Jewish Hospital of St. Louis closely follows that of Howe,¹⁰ except that serum is used instead of plasma. This method in skillful hands gives trustworthy results and is rivaled only by refractometric methods and possibly by the new one of Campbell and Hanna.¹¹

Different observers have varied somewhat widely in their figures of normal percentages of albumen and globulin in plasma and serum. This variation may

in part be due to differences in technique but may also be due to a variation in the clinical material used. Peters and Eisenman¹² give as their normal values: total serum protein 6 to 8 per cent, serum albumen 4 to 5 per cent, and serum globulin 1.4 to 3 per cent. Other observers vary in either direction. The suggestion of Starlinger and Winands¹³ that only values of serum globulin greater than 4.0 per cent be considered indicative of hyperglobulinemia probably deserves acceptance.

Serum globulin itself appears to be a mixture of proteins and of these euglobulin is apparently the one that has clinical significance. The highest normal value found by Ling¹⁴ was 0.333 per cent, and it is safe to say that, if the determinations are made by Howe's method, any euglobulin value above 0.5 per cent is unusual and anything over 0.75 per cent is definitely pathologic. In the cases reported here much higher figures were found.

Convenient qualitative indications of an excess of euglobulin are furnished by the dilution test and the formol-gel test. The former is done by adding 9 c.c. distilled water to 1 c.c. serum. A cloudy solution in which upon standing a flocculent precipitate forms indicates an excess of euglobulin. In the formol-gel test one drop of 30 per cent formalin is added to 1 c.c. clear serum in a small test tube and mixed by gentle shaking. In the presence of a considerable excess of euglobulin the mixture gels and becomes opaque within a few hours, sometimes within a few minutes.

Infectious diseases are usually accompanied by a rise in the percentage of serum globulin and often by a fall in serum albumen. Usually these variations are relatively slight, so that there is no inversion of the albumen-globulin ratio. In certain diseases, however, the rise in the globulin value may be so great that it not only exceeds the critical 4.0 per cent but may be relatively enormous so that, in spite of a considerable fall in the percentage of serum albumen, the amount of total serum protein far exceeds the normal.

Kala azar was the first infection in which extraordinarily high values of serum globulin were observed and a marked hyperglobulinemia is apparently a constant finding in this disease. Wn's case¹⁵ in which the total protein values were 10.52 per cent with 7.06 per cent globulin apparently represents a record in this disease. Ordinarily the serum globulin does not exceed 5.0 per cent. The euglobulin values are usually between 2 per cent and 3 per cent.

In syphilis, especially in the florid secondary cases, there is often a fall in the serum albumen and a corresponding rise in serum globulin. The latter is usually moderate¹⁶ but may reach 5.0 per cent and at least once¹⁷ has been reported as 6.5 per cent.

The highest globulin values ever found in blood serum have occurred in multiple myeloma. Thus, in one case reported by Shirer, Duncan, and Haden,¹⁸ the serum protein amounted to 13.78 per cent and the globulin to 11.34 per cent. In all cases of multiple myeloma with hyperproteinemia the high protein value has been due to a substance supposed to be globulin.

Just what this globulin-like substance is remains uncertain. It is salted out of the serum with the euglobulin, and some observers¹⁹ believe it definitely to be euglobulin. Others, especially Shirer, Duncan, and Haden¹⁸ and Cantarow,²⁰ be-

lieve it to be the Bence-Jones protein that is so often found in the urine in this disease. Our own very limited experience with this substance indicates that it is neither euglobulin nor Bence-Jones protein, and perhaps not even a true globulin.

Within the past few years two cases were observed, one at the Jewish Hospital and one at Barnes Hospital of St. Louis, which fall more readily into the syndrome of Still's disease, in its more inclusive form, than into any other category, and whose serum globulin and euglobulin values reached a level hitherto observed only in kala azar and possibly in multiple myeloma.

CASE 1.—L. S., 18 years old, though looking like a child of 12, entered Barnes Hospital in February, 1935. For over a year he had had a migrating polyarthritis and this had left behind a swelling of the elbows and knees, exaggerated by the muscular atrophy of the arms and legs. There was some limitation of motion. On admission his emaciation and pallor were extreme and there was evidence of pericardial effusion. The spleen was palpable. The liver was enlarged and tender, suggesting chronic passive congestion. There was a generalized lymphadenopathy, but the glands were not very large. During the three months he was in hospital he ran an irregular fever, sometimes exceeding 104° ; the temperature was never normal for more than twenty-four hours at a time. The arthritis flared up from time to time, but there were intervals during which he was quite free from pain. His blood showed a marked secondary anemia, requiring repeated blood transfusions. The white cells varied from a slight leucocytosis (12,250) to a moderate leucopenia (5,100); the hemogram constantly showed a shift to the left. The Kahn test was negative; the serum calcium was 9.3 mg. per cent and serum phosphorus 4.5 per cent. Neither blood serum nor urine contained any Bence-Jones protein.

The pericardium was aspirated and 35 c.c. clear fluid were withdrawn; culture and guinea pig inoculation were negative. Repeated blood cultures were negative as were also all the usual agglutination tests.

He remained in hospital until May 17, the clinical picture remaining unchanged and the fever persistent. After a few months at home, he reentered in August on account of dyspnea and thoracic pain. There was again evidence of pericardial effusion and 110 c.c. turbid fluid were withdrawn. The cells consisted chiefly of neutrophils with many monocytes and rare eosinophiles; culture of the fluid showed no growth. The anemia was again extreme with a more marked leucopenia (4600) and a normal hemogram. The spleen was larger than before, the liver also somewhat enlarged. The Congo red test for amyloidosis was negative.

On October 2, he insisted upon leaving for his home in the country, where he died two days later. No autopsy could be obtained.

The blood serum had always shown marked flocculation on dilution with distilled water; the formol-gel test always produced milkiness and jelling within five minutes. The serum protein values were as follows:

DATE	TOTAL PROTEIN	SERUM ALBUMEN	SERUM GLOBULIN	EUGLOBULIN	PSEUDO-GLOBULIN
3/ 2/35	9.71 per cent	2.38 per cent	7.33 per cent	2.59 per cent	4.74 per cent
4/17/35	14.1	2.4	11.7		
4/29/35	11.4	2.3	9.1		
5/18/35	10.36	2.14	8.22	3.67	4.55
8/ 8/35	8.4	1.4	7.0		
9/12/35	7.25	1.76	5.59	1.67	3.92

CASE 2.—F. R., aged 59, entered the Jewish Hospital of St. Louis in September, 1934, complaining of loss of weight and increasing weakness. During the previous summer she had suffered much from arthritis, involving chiefly the left shoulder and elbow. The joints were

painful and showed swelling, redness, tenderness, and limitation of motion. When she entered the hospital the manifestations had pretty well subsided, but there was still some thickening about the joints and some arthritis of the cervical spine. She was found to be running an irregular fever, at first only occasionally reaching 102°, but later during her stay in hospital exceeding this level nearly every evening. Physical examination showed a systolic cardiac murmur that had been present at a previous admission nine years before, a moderate cervical, axillary and inguinal adenopathy, a slightly enlarged liver, and a greatly enlarged spleen. The blood count on admission showed red cells 4,400,000, hemoglobin 90 per cent, white cells 3,900, hemogram

Ba	Co	My	Ju	St	Se	Ly	Mo
1	1	0	0	17	50	27	4

During her three months' stay in hospital the white count was never quite so low again but exceeded 6,500 only once, while a moderate secondary anemia made its appearance. Repeated blood cultures were negative. The Kohn test was sometimes positive, sometimes negative, the Wassermann, when not anticomplementary, was negative, there was nothing in the history suggesting syphilis. All the routine agglutination tests were negative, as were intradermal tests for tuberculosis and brucellosis. Spleen puncture and sternal marrow biopsy gave normal findings. The urine, at first normal, later contained albumen, cast, and red cells. Repeated examination of blood and urine was negative for Bence Jones protein.

In January, 1935, the arthritis, which had not been marked for several months, flared up again with pain, tenderness, redness, and swelling of the right ankle. It subsided after some weeks, but recurred again two months later.

During all of this time until her death in May she continued to run a high daily fever of a septic type. The blood picture showed a secondary anemia, progressively more severe, and a moderate leucopenia. A typical count was that of March 13, 1935: red cells 2,810,000, hemoglobin 63 per cent, white cells 7,500, hemogram

Ba	Co	My	Ju	St	Se	Ly	Mo
0	2	0	0	6	34	56	2

The platelet count was 180,000 toward the end of her illness; many small petechiae were seen scattered over the entire body. Repeated blood cultures were negative. The spleen slowly grew in size, being firm and smooth, and finally extending some 11 cm. below the costal margin. The very moderate adenopathy remained unchanged. The liver was always distinctly palpable. There was never any free fluid in the abdominal cavity.

In May, 1935, seven months after entering the hospital, she died in coma with a terminal pneumonia. Unfortunately autopsy was refused.

The blood serum, like that of the first case, showed a heavy flocculation upon dilution with distilled water, indicating the presence of a large excess of euglobulin. All sera except the last solidified and became opaque promptly upon the addition of formal. The serum protein values were as follows:

DATE	TOTAL PROTEIN	SERUM ALBUMEN	SERUM GLOBULIN	EUGLOBULIN	PSEUDO GLOBULIN
11/ 2/34	77 per cent	32 per cent	45 per cent	23 per cent	22 per cent
11/ 7/34	90	39	51	12	39
12/ 4/34	71	33	38	11	27
2/16/35	787	210	577	224	353
5/21/35	819	390	429	083	346

COMMENT

There may well be a difference of opinion regarding the propriety of considering these two cases as examples of Sull's disease, not only because the delimitation of this syndrome is itself somewhat vague. In both cases Banti's

disease was considered, but the continuous high fever, the arthritis, the adenopathy and the total absence of aseities did not fit this picture. A complete x-ray study of the bones should have been done to exclude multiple myeloma, but nothing in the clinical picture suggested this diagnosis, nor did blood or urine ever contain Bence-Jones protein. The two cases seem worth reporting because it may be that a study of the blood proteins may help us to a further knowledge of those very interesting cases that have been grouped together under the name of Chauffard-Still syndrome.

SUMMARY

Two cases are reported characterized by arthritis, adenopathy, splenomegaly, and long-continued high fever. For want of a better diagnosis they are included in the so-called Chauffard-Still syndrome.

Both cases show hyperglobulinemia, with an extremely high euglobulin percentage.

I desire to express my thanks to Dr. David P. Barr, Head of the Department of Medicine of Washington University, for permission to report the first case; and to Dr. Michael Somogyi, biochemist at the Jewish Hospital of St. Louis, for the blood protein determinations.

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THE INFLUENCE OF CALCIUM SALTS ON DIGITALIS ACTION*

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IT IS held quite widely by clinicians and research workers that intravenous calcium medication is dangerous, especially when strong concentrations are used, and exceptionally so in digitalized patients. The intravenous use of calcium salts is extensive. It has been used in the treatment of lead poisoning, after parathyroidectomy, in tetany, tuberculosis, nephritis with edema, after hemorrhage, in heart disease, in allergic diseases, such as eczema, urticaria, angioneurotic edema, asthma, hay fever, etc., deficient calcification of the bones, to relieve the pain in inoperable cancer, in alkalosis, in jaundice preliminary to operations, etc.

Bower and Mengle¹ report two deaths in patients following the intramuscular administration of digitalis and the intravenous injection of 10 cc of 10 per cent calcium gluconate in one case, and 10 cc of 10 per cent calcium chloride in another. Lloyd² reports that rapid intravenous injection of calcium chloride (10 per cent) is dangerous, the electrocardiogram showing what he interpreted as sino auricular block. Berlner³ found that the intravenous injection of 10 cc 20 per cent calcium gluconate in man produced electrocardiogram changes which indicated damage to the heart. He states that when injected slowly and in proper amounts, calcium salts are harmless, but when administered rapidly they are potentially dangerous. Lieberman,⁴ working with dogs, found that when calcium gluconate is given slowly (about 0.5 cc per minute of about 12 per cent) about ten times the amount could be injected as if the rate of injection was 1 cc per minute.

Calcium salts injected into the blood stream affect the heart somewhat like digitalis. Gold and Edwards⁵ found a synergism between calcium and ouabain in dogs. Lieberman found only an additive effect of calcium with digitalis and with scillaren B. Nyiri and DuBois,⁶ working with frogs, found that calcium, above a certain concentration, intensifies the action of digitalis. Weichmann⁷ also found that in the isolated frog's heart calcium ions increase the action of digitalis. Loewi⁸ accepts the synergistic action of calcium and digitalis and explains the synergism by stating that digitalis sensitizes the heart toward calcium ions. Combating the explanation of Loewi, Forscher,⁹ also Fischer,¹⁰ claims that digitalis exerts a specific action independent of the calcium. Fischer offers the following explanation. The heart is sensitized by digitoxin for certain kinds of stimuli, among others for calcium ions, and there is a reciprocal synergism between calcium and digitalis. Schuntermann¹¹

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thinks the synergism is due to the condition and amount of electrolytes in the tissues (electrolyte milieu).

Some of the experiments upon which the above opinions are based were on the isolated heart. Some were on intact animals. Each method has its advantages and disadvantages, but it would seem more physiologic to use intact animals, and for comparison with man, warm-blooded animals would seem preferable. Colldahl¹² used intact tadpoles, and, to insure better absorption, treated them with the glucoside cyclamin, after the method of Overton. By this method a definite constant concentration of the drug may be kept in the blood. This method has the advantage that a known concentration of calcium as well as digitoxin can be kept in the circulation. Colldahl found that digitalis or increased calcium content of the blood caused a decrease in the heart rate. A digitoxin concentration of 1:800,000 had an action similar to 1:500 of calcium chloride. Quite contrary to most of the reported work, however, he found that calcium lessens the toxic action of digitoxin. He states that his results correspond with certain clinical observations of which he quotes the following:

Billigheimer¹³ thinks that the action of digitoxin on the heart is due to the liberation of calcium ions, and states that cases of tetany (calcium deficiency) with sound heart, tolerate little digitalis. On the other hand, cases of encephalitis in which the blood calcium content is quite high tolerate enormous doses of digitalis.

Singer¹⁴ says that "while calcium increases and hastens the action of digitalis on the heart, it lessens the untoward actions of digitalis on the parasympathetic system. It is both the whip and the rein for digitalis." Seelig¹⁵ calls attention to the possible ill effects on the heart by 10 per cent calcium chloride intravenously, and recommends that 1 per cent be used and injected slowly. This suggestion corresponds to the findings of Lloyd, Lieberman, and to our own, and we believe it has not received the attention it merits.

Quite recently Nahum and Hoff¹⁶ have studied the effect of calcium on the digitalized heart. They used rabbits in their work and studied particularly the electrocardiogram changes produced. They found that digitalized rabbits tolerated as much calcium as controls, and concluded that in the normal nonanesthetized rabbit heart the effects of calcium and digitalis are not additive. Their work is not quite comparable with ours since after "arrest" of the heart with calcium in controls, they were able to use the animals again with digitalis. In clinical cases where death occurred after digitalis and calcium, they incline to the opinion that it was entirely due to digitalis. We think it was more likely due to the too rapid injection of the calcium salts.

The work of Nahum and Hoff has been criticized adversely by Gold and Kwit¹⁷ on the fact that the dose of digitalis as used by Nahum and Hoff, and referred to as 50 to 75 per cent of the calculated lethal dose, was only about 6 to 9 per cent of the lethal dose for the rabbit, the lethal dose of digitalis for the rabbit being about 8 times as much as for the cat.

EXPERIMENTS

Dogs were used in all our experiments. They were anesthetized with pentobarbital sodium, 35 mg per kg body weight, given intraperitoneally. This drug as given affects the heart but slightly, and, therefore, is well suited to study drugs that kill by affecting the heart. Injection of the calcium salts was into the femoral vein, each injection, unless specified, being made in about one minute, and injections were repeated at five minute intervals.

FATAL DOSE OF CALCIUM CHLORIDE

WT OF DOG IN KG	PER CENT CaCl ₂	INJECTIONS (CC) EVERY 5 MIN	TOTAL DOSE CC	FATAL DOSE CaCl ₂ PER KG
10.9	3.7	10.0	36.0	1.222
8.6	5.0	15.0	15.0	0.880
6.0	5.0	10.0	8.0	0.666
23.0	5.0	12.5	27.5	0.550
23.2	5.0	11.6	44.2	0.960
15.0	5.0	10.0	28.0	0.933
13.6	5.0	20.0	8.0	0.300
9.5	10.0	10.0 slowly	6.0	0.630
20.9	10.0	10.0 very slowly	16.0	0.765

From the above experiments it is concluded that the amount of calcium that may be injected before death occurs depends largely on the rate of injection. Twenty cubic centimeters of 5 per cent injected rapidly may kill at one injection, whereas 400 cc, or over one gram per kilogram, may be given if injected slowly.

FATAL DOSE OF CALCIUM GLUCONATE

WT OF DOG IN KG	% Ca GLUCO NATE IN SALINE	RATE (CC) EVERY 5 MIN	TOTAL (CC)	GM	EQ TO CaCl ₂ PER KG
8.0	10	5.0	25.0	3.08	0.740
13.2	10	10	45.0	3.00	0.740

While these large doses may be injected, three injections of 20 cc each killed a 10 kg dog, and one such injection may kill a smaller animal. Two or 3 per cent solutions of calcium gluconate may be injected ad libitum and kill mainly by volume effect.

FATAL DOSE OF DIGITALIS

In dogs anesthetized as we have done, with pentobarbital, 35 mg per kg, the fatal dose of the digitalis was 1 cc of the tincture per kg. In fact the dose is so uniform that we think that this method may be used to standardize digitalis. The variation we have found in a number of healthy dogs is not over 10 per cent from this figure. The number of animals used in this case,⁹ while sufficient for the present purpose, is not enough to say that the method may be used for standardization. We are at work on this problem.

EXPERIMENTS WITH DIGITALIS ALONE

A strength corresponding to the 10 per cent tincture in 25 per cent alcohol was used, and 1 cc injected into the femoral vein every five minutes.

WT. OF DOG IN KG.	RATE (C.C.) EVERY 5 MIN.	FATAL DOSE (C.C.) 10% TINCTURE
7.3	1.0	8
6.0	1.0	6
12.0	1.0	11
8.2	1.0	8
7.7	2.0	8
16.6	2.0	18
14.6	2.0	16
13.6	1.0	13
10.9	1.0	10

DIGITALIS GIVEN FIRST, 1 C.C. EVERY FIVE MINUTES, FOLLOWED BY CALCIUM GLUCONATE

WT. OF DOG IN KG.	DIGITALIS (C.C.) 10%	Ca GLUCONATE (C.C.) 10%	FATAL DOSE (%) DIGITALIS	Ca GLUCONATE
10.0	6	120	60	40
6.6	4	100	52	46
13.0	12	30	92	8

Calcium Gluconate Given Before Digitalis

10.0	9	60	90	20
8.2	8	6	97	2

Calcium Gluconate (2%), 1 c.c. Injections, Alternated with Digitalis

13.0	11	11	90	0.4
10.0	11	11	110	0.6

In another dog, 8 kg., rapid injection of 25 c.c. 10 per cent calcium gluconate, after 50 per cent of the fatal dose of digitalis, killed almost immediately. Slow injection causes no such effect. These experiments show that calcium gluconate in larger or smaller dose has no perceptible action on digitalis, other than an additive toxic effect.

DIGITALIS WITH CALCIUM CHLORIDE

Since the toxic dose of calcium chloride varies with the rate of injection, we have tried to give the injections at a uniform rate. The toxic dose of calcium chloride for purpose of calculation is taken at 550 mg. per kg. body weight. This was the minimum fatal dose found when we injected 5 per cent calcium chloride at a rate of 0.5 c.c. per kg. every five minutes. If injected more slowly, this figure may not be correct, but it will serve for purposes of comparison, and if any striking effect of giving digitalis and calcium chloride together is an actuality, it should demonstrate it. Because it has been stated by some that digitalization is sometimes rendered less toxic by calcium chloride, we have varied the doses and concentrations of the calcium.

EFFECT OF SUDDEN CHANGES OF CALCIUM CONCENTRATION ON THE HEART

Carl Schmidt (quoted from Mathews' *Physiological Chemistry*) gives the calcium concentration of the blood at 0.0193 per cent calcium phosphate. This is equivalent to 0.007 per cent calcium. Since the volume of blood in a person weighing 70 kg. is about 5,500 c.c., the total amount of calcium would be 0.355 grams.

DIGITALIS ALTERNATED WITH CaCl_2
 DIGITALIS WITH 0.5% CaCl_2 , ALTERNATE INJECTIONS OF 1 c.c. DIGITALIS, 0.5 c.c. CaCl_2

WT. OF DOG IN KG.	DIGITALIS	CaCl_2	FATAL DOSE (%) DIGITALIS	CaCl_2
8.4	9.0	4	107	0.2
<i>Digitalis with 1% CaCl_2, Alternate Injections</i>				
8.0	9.0	9	110	0.5
<i>Digitalis Given First, Followed by 5% Calcium Chloride 0.5 c.c. per kg.</i>				
18.2	9.1	118	50	55.0
20.0	10.0	190	50	44.0
26.0	14.0	60	54	20.0
The rapid injection in the last case (15 c.c. at dose) was a factor in death.				
17.3	9.0	81	53	43.0
<i>Alternating Doses of Digitalis and 5% Calcium Chloride</i>				
20.9	13.0	84	62	44.0

Each 10 c.c. of a 10 per cent solution of calcium chloride contains 0.36 gm. of calcium. Consequently the intravenous injection of 10 c.c. would double the normal calcium concentration immediately. Since this may reach the heart in several times this concentration, it may cause immediate stoppage. The effect is directly on the muscle, since we have found it is not prevented by atropine. The effect, however, on the tracing is similar to that which occurs after stimulation of the vagus. Rapid injection of many drugs may cause a stoppage of the heart, which may be temporary or permanent, and for lack of knowledge of the detailed mechanism is described as a "shock" effect; in this case it is apparently due to sudden change of the electrolyte milieu. Such reactions emphasize the importance of slow injections of any intravenous medicament. In the case of calcium chloride, a 10 c.c. dose should occupy at least two minutes, at a uniform rate.

A recent study by Hoff and Nahum,¹⁸ using the electrocardiogram, shows that 1 to 2 c.c. of a 10 per cent calcium chloride solution injected at the rate of 2 c.c. per minute in unanesthetized rabbits produces a slowing of the heart, auriculoventricular delay, and auricular fibrillation. Larger doses cause extrasystoles from many foci, periodic cardiac arrest alternating with ectopic ventricular beats (Luciani periods), and cardiac arrest.

From the above experiments the following conclusions may be drawn:

1. Calcium salts affect the heart in a manner similar to digitalis, and their action is additive.
2. Calcium salts may be injected intravenously in quite large amounts if given slowly. The rapid injection of 5 per cent is potentially dangerous, either in presence or absence of digitalization.
3. There is no specific danger in the slow administration of calcium after digitalis other than an additive effect.

4. Calcium gluconate is safer than calcium chloride for intravenous use only because it contains less calcium. A saturated water solution (about 3 per cent) may be injected at the rate of 10 c.c. per minute without much danger. One-half this rate, however, is recommended.

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LABORATORY METHODS

THE MICRODETERMINATION OF CALCIUM IN WATER*

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FROM time to time most laboratories are called upon to determine the calcium content of water, or they may desire to check upon the efficiency of the operation of a water softener or still. Since the methods given in *Standard Methods of Water Analysis*¹ require considerable time, it occurred to us that the Clark Collip modification of the Kramer Tisdall method might be employed for this purpose. This method is commonly used for the determination of calcium in blood serum, but, as far as we know, it has never been suggested for the determination of calcium in water.

METHOD

The following technique (as given by Mattice²) was employed. 5 ml. of water and 1 ml. of saturated ammonium oxalate are placed in a conical (15 ml.) centrifuge tube. The contents are mixed by gentle agitation and allowed to stand for two to four hours (Mattice² recommends that, when convenient, they be allowed to stand overnight in the icebox). At the end of the period, the tubes are centrifuged and the precipitate is washed twice with dilute ammonia, then dissolved in 2 ml. of approximately N H_2SO_4 and the solution after heating is titrated with 0.01 N $KMnO_4$ in the usual manner. A blank determination is conducted by adding 1 ml. of the ammonium oxalate solution to 5 ml. of distilled water.

RESULTS

In order to try out the method a stock calcium solution was prepared and standardized according to *Standard Methods of Water Analysis*. The solution, when diluted, contained 0.09 mg. calcium per ml. This was added to distilled water in varying amounts and the calcium was determined by the method given above. The results are given in Table I. Each figure given is the average of closely agreeing duplicate determinations made upon different days. In every case, the "blank" has been deducted from the total amount of $KMnO_4$ used in the titrations.

*From the Arizona State Laboratory, Tucson.
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TABLE I
RECOVERY OF CALCIUM ADDED TO DISTILLED WATER
1 ML. CA SOLUTION \approx 0.09 MG. CA

ML. CA SOLUTION	ML. DISTILLED H ₂ O	MG. CA ADDED	ML. 0.01 KMnO ₄	MG. CA RECOVERED
1	4	0.09	0.45	0.09
2	3	0.18	0.90	0.18
3	2	0.27	1.40	0.28
4	1	0.36	1.80	0.36
5	0	0.45	2.25	0.45

Table I shows that excellent recoveries were obtained, so the experiment was repeated by adding a calcium solution of known strength to tap water. Many analyses of the tap water according to *Standard Methods of Water Analysis*¹ have shown that it varies between 40 to 45 parts per million of calcium with an average of about 42 parts per million.

TABLE II
RECOVERED OF CA ADDED TO TAP WATER
1 ML. CA SOLUTION \approx 0.09 MG. CA

ML. CA SOLUTION	ML. TAP WATER	MG. CA PRESENT	ML. 0.01 N KMnO ₄	MG. CA RECOVERED
0	5	0.21	1.05	0.210
1	4	0.258	1.25	0.250
2	3	0.306	1.55	0.310
3	2	0.354	1.80	0.360
4	1	0.402	2.05	0.410
5	0	0.450	2.30	0.460

The recoveries obtained agree well with the amounts of Calcium known to be present. In order to determine how sensitive the method is a more dilute solution of Calcium was added to distilled water. The results are given in Table III.

TABLE III
RECOVERY OF SMALL AMOUNTS OF CALCIUM ADDED TO DISTILLED WATER
1 ML. CA SOLUTION \approx 0.01 MG. CA

ML. CA SOLUTION	ML. DISTILLED H ₂ O	MG. CA PRESENT	ML. 0.01 N KMnO ₄	MG. CA RECOVERED
1	4	0.01	0.05	0.01
2	3	0.02	0.10	0.02
3	2	0.03	0.15	0.03
4	1	0.04	0.2	0.04
5	0	0.05	0.3	0.06

Although we secured excellent recoveries of calcium, it is not recommended that the method be applied to waters which would require less than 0.5 ml. of 0.01 N KMnO₄. We have tried the use of more dilute permanganate (0.005 and 0.001 N), but find that due to the dilution, considerable errors are introduced; excessive amounts of the permanganate are required to give the characteristic end point.

If the water contains less than 16 to 20 parts per million of Ca, 20 ml should be evaporated to a volume of 10 ml and the determination may be made upon this concentrate. If the calcium content is large, use a smaller sample and sufficient distilled water to bring the volume to 5 ml.

To calculate parts per million

$$\begin{aligned} \text{If sample} &= 5 \text{ ml and } \text{KMnO}_4 \text{ is } 0.01 \text{ N} \\ (\text{ml } \text{KMnO}_4 - \text{blank}) \times 0.2 \times 200 &= \text{ppm Ca} \\ (\text{ml } \text{KMnO}_4 - \text{blank}) \times 40 &= \text{ppm} \end{aligned}$$

If other volumes of sample are used, multiply the volume of KMnO_4 used (less blank) by the corresponding factor

VOLUME OF SAMPLE ML	FACTOR
1	200
2	100
3	66.6
4	50

SUMMARY

The Clark Collip modification of the Kramer-Tisdall method may be applied directly to waters of calcium content greater than 16 to 20 parts per million. Any technician doing blood chemistry should be already familiar with the method and should have all the necessary reagents and equipment. The method has given excellent results in this laboratory.

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STANDARDIZATION OF THE IOLIN MALMROS MICRO BLOOD SUGAR METHOD FOR CAPILLARY AND VENOUS BLOOD AND ITS ADAPTION TO THE PHOTOELECTRIC PHOTOMETER*

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FREQUENT errors occur in visual colorimetry through variation in color sensitivity among observers and through fatigue in the observers. Several colorimetric methods have been introduced recently which utilize photoelectric cells to improve accuracy. In 1935 Goudsmit and Summerson¹ introduced a variable layer photoelectric comparison photometer based on the principle of the Duboseq colorimeter. Their method makes use of photoelectric cells for measuring the intensity of color in each cup independently. Differences in

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turbidity are also recorded as with any photometer. This flexible instrument with its improved accuracy should prove a distinct advantage in routine determination of blood sugars by such a method as the Folin-Malmros.

The Folin and Malmros² modification for determination of capillary blood sugar has advantages which makes it a valuable procedure. It is not only simple and accurate but has the additional advantage of slow fading of color. This slow fading permits a long series of determinations which can be read without appreciable loss. For these reasons, their method was peculiarly adaptable to the study we were making.

It became evident, however, in determining blood sugars in this way that certain factors must be investigated before it could be adapted to the photo-electric colorimeter. Two serious disadvantages were encountered among these factors, which had to be corrected. The first was the complicating one of a variable multicolored solution, i.e., the color of the Prussian blue and of the potassium ferrieyanide. Folin and Malmros suggested the use of a yellow filter with the visual colorimeter to eliminate the color of the ferrieyanide. A second disadvantage was a progressive loss from the calculated amount of sugar as the concentration of the sugar solution was increased. These two difficulties will be discussed later, as well as such other variables as time of heating, intensity of the light in the colorimeter, and the stability of the Prussian blue.

TABLE I

DUPLICATE DETERMINATIONS MADE ON CAPILLARY BLOODS DURING ROUTINE GLUCOSE TOLERANCE TEST AND ON KNOWN GLUCOSE SOLUTIONS

CAPILLARY BLOODS GLUCOSE TOLERANCE TESTS			KNOWN GLUCOSE SOLUTION	
Case S. K.	Case L. J.	Case A. F.	7/19/37	7/20/37
98.0	105	102	144	144
98.5		104		
144.9	134	159	185	185
147.0	137	160	215	215
143.9	200	141	264	264
143.9	200	143	299	295
178.6	179	111	345	345
178.6	178	113	375	378
137.9	135	116	409	400
140.8	136	117	418	418

TECHNIQUE

Capillary and oxalated venous blood were obtained in the usual way. The technique of the method used is essentially that recommended by Folin and Malmros, with the exception of the length of time for heating. Folin and Malmros gave directions for heating the tubes eight minutes. In this study the tubes were placed in the water-bath for twenty minutes. A 0.001 per cent glucose solution was used as a standard. The directions for use of the colorimeter given by Goudsmit and Summerson were followed. A yellow glass filter (supplied by Klett) proved to be of advantage. The deeply colored solutions in the Folin-Malmros method made a high intensity of light desirable, i.e., the A.C. transformer set at 6, and the galvanometer switch set at the No. 2 position. Although a

lower intensity of light gave essentially identical readings as the higher, the higher intensity increased the sensitivity of the colorimeter. It further resulted in a saving of time by making it possible to approach the null point of the galvanometer more rapidly. Two or more readings were made by racking the cup up or down. However, after some experience with the instrument, only one reading was necessary to insure accuracy. With proper care, 15 to 20 blood sugar determinations may be read without any loss of accuracy due to shifting of the instrument or to fading of the color. Scrupulous attention to details of operation and cleanliness of the instrument must be maintained.

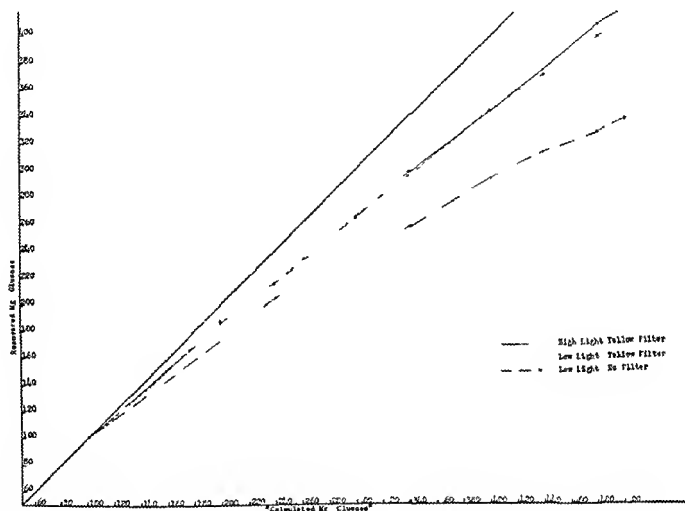


Fig. 1—Recovery experiment on known glucose solutions showing effect of high light and low light intensity with a filter and low light intensity without a filter

DISCUSSION

A very satisfactory accuracy may be obtained with the use of the Folin Malmeos method when read with the photoelectric colorimeter. This is demonstrated in Table I, which shows a series of known glucose solutions determined on successive days. This same table shows a series of duplicate capillary blood sugars, taken during the course of routine dextrose tolerance tests. This degree of accuracy was difficult to approach with the visual colorimeter and especially so when the readings were made by different observers. Identical readings with the photoelectric colorimeter were readily obtained by different observers.

When variations occur in results obtained with the photoelectric colorimeter, they are significant of pipetting inaccuracies.

As indicated before, certain standards were selected somewhat arbitrarily because they offered advantages in constancy as well as speed of operation. With a high intensity of light no appreciable difference was noted whether the galvanometer switch was set at position 1 or 2. However, at position 1, somewhat lower readings were obtained when the intensity of the light was diminished. Such lower readings were especially characteristic with higher concentration of sugar. Fig. 1 demonstrates recoveries of a series of known solutions of glucose when readings are made with standard technique, with low light intensity, and without the yellow filter. It should be noted that the first two series of readings are almost identical except at higher concentrations when they tend to be a little lower with the low light. The percentage of difference, however, is minimal. Readings without the yellow filter are markedly lower than with the filter, and become progressively lower with the higher concentration of sugar. This difference is probably due to the progressively decreased yellow color of the ferrieyanide solution as compared with the standard. A disturbance in the proportionality of the color of the solutions is reflected in the readings and produces a marked variation from the calculated readings. When the filter is used, this variation is largely obviated.

Studies made on the effect of the duration of heating indicate that eight to ten minutes is probably sufficient to complete the reduction of the ferrieyanide to ferrocyanide. Twenty minutes' heating was used routinely, because, as suggested by Somogyi,³ eight minutes may not give complete reduction. We found when blood filtrates were used the recoveries after eight minutes seemed to be lower and less constant than those obtained with twenty minutes' heating.

TABLE II

SHOWING THE STABILITY OF THE PROPORTIONALITY OF THE COLOR DEVELOPED IN THE FOLIN-MALMROS MODIFICATION CAPILLARY BLOOD SUGAR METHOD

NUMBER	READING ON THE COLORIMETER SCALE		
	IMMEDIATELY	HALF-HOUR	ONE HOUR
1	35.3	35.2	35.2
2	19.6	19.7	19.7
3	14.0	14.1	14.1
4	10.75	10.8	10.8
5	9.2	9.3	9.3
6	7.3	7.4	7.3
7	6.65	6.7	6.7
8	5.7	5.7	5.7
9	5.2	5.25	5.3
10	4.9	4.9	4.95
11	4.7	4.75	4.75

In Table II the stability of the proportionality of the Prussian blue is illustrated. Readings made immediately, in one-half hour and in one hour, show no evidence of fading and give essentially the same readings. This factor was significant when fifteen to twenty determinations were made in a given series.

Even after these variables were corrected and standardized, the readings obtained with the photoelectric cell colorimeter still did not approach the calculated readings and gave lower values than those obtained with the visual

colorimeter. The readings were consistent and produced a straight line curve when plotted on logarithmic paper. The depth of the color apparently did not follow Beer's law when applied to the response of the photoelectric cell. This discrepancy may be due to failure of quantitative production of the color or to interference by colloidal particles introduced with the non gum ghatti solution. In either case the factor was a constant for which a calibration curve (Fig 2) was made. This curve was made for the 0.001 per cent standard. A 0.002 per

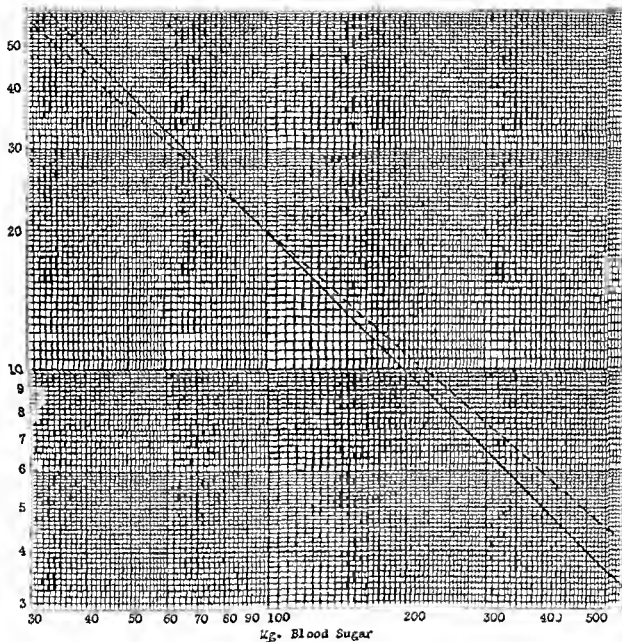


Fig 2—Calibration curve for 0.001 per cent standard glucose solution

cent sugar standard may be used which will give accurate readings up to 250 mg per cent of blood sugar without correction. This standard was discarded because it necessitated readjustment of the instrument with the change in standard. With the use of the calibration curve, accurate readings may be obtained as high as 500 mg per cent, using only the 0.001 per cent standard. Fig 2 shows the calibration curve. Table III shows readings on several blood sugar determinations corrected by using the calibration curve. When recoveries with the 0.001 per cent standard are corrected with the calibration curve, they will superimpose on recoveries with the double standard (0.002 per cent) up to a level of 250

TABLE III
SHOWING USE OF THE CALIBRATION CURVE IN HIGHER CONCENTRATIONS OF GLUCOSE

SPECIMEN	CALCULATED	RECOVERY	CORRECTED	DIFFERENCE
A. Mixture of 5 blood specimens		171	182	
B. 0.1 c.c. of (1 c.c. of A plus 1 c.c. of 0.33 per cent glucose)	258	232	261	plus 3
C. 0.1 c.c. of (1 c.c. of A plus 1 c.c. of 0.5 per cent glucose)	342	300	344	plus 2
D. 0.1 c.c. of (1 c.c. of A plus 1 c.c. of 0.67 per cent glucose)	425	361	425	minus 1
E. 0.1 c.c. of (1 c.c. of A plus 1 c.c. of 1 per cent glucose)	591	470	588	minus 3

mg. per cent. Above this level, correction must be made with the double standard also. We feel that the use of the single standard (0.001 per cent) is much simpler and gives as great a degree of accuracy as the use of several standards.

SUMMARY

The variable layer photoelectric colorimeter is adaptable to routine sugar determinations by the Folin-Malmros capillary method. The method is accurate and simple. A yellow filter is advantageous for securing consistent results. The readings with the colorimeter do not follow Beer's law, and a calibration curve is necessary for accurate results. A standardized technique permits a high degree of accuracy and makes feasible the use of the method for routine determinations of blood sugar.

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A METHOD FOR STAINING RICKETTSIA IN SECTIONS^{*}

PROF. DR. SADAMI OCHI, SAPPORO, JAPAN

GIEMSA'S stain is generally used in the study of rickettsia in smear preparations. In well-made smears stained by the Giemsa method, one can demonstrate massively grouped rickettsia very well. In such smear preparations it is readily observed that the rickettsia are very definitely intracellular. Furthermore, Giemsa stain is used for the demonstration of rickettsia in tissues. Da Rocha-Lima has used Giemsa to stain *Rickettsia prowazeki* in sections from typhus: "the best stains are obtained in forty-eight hours, using 20 c.c. distilled water + 10 drops Giemsa solution, changing the staining solution every twenty-four hours. If one wishes rapid staining, 20 c.c. of water and 10 drops of Giemsa are used, changing the stain once or twice, for a period of two to three

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hours. The staining is made by flooding the slide, in a horizontal position" (Handb d path Mikroorganismen 9: 926, 1929)

Notwithstanding that this method of staining with Giemsa has been proposed, it appears that staining of sections in the study of rickettsia is infrequently used. Possibly there is some valid ground for this. Nevertheless, if the manipulation of section staining is simple, the microbiologic study of stained sections might be important in rickettsia investigations, as in other, similar, cases.

In fact, well stained sections could be very useful in microbiologic investigations. If, for example, some sort of colored bodies should be observed in stained sections, sufficient study could be made by serial sections to determine if they are, or are not, microbiologic in nature. It is self evident that one cannot determine by stained smears whether the colored bodies in the tissue are a pathologic reaction product of the tissues or actual pathogenic microorganisms. But in stained sections one can observe simultaneously, and compare, the tissue changes and the stained bodies. Therefore, the examination of stained sections becomes more and more essential in such microbiologic studies, particularly if such bodies are readily stainable in sections.

The history with reference to the rickettsia is hereby omitted. Rickettsia have previously been found in cases of typhus. They have also been discovered, in Japan, in cases of tsutsugamushi disease. There are many reports of the detection of rickettsia in other diseases. H. Da Rocha Lima says "Some rickettsia, and among them *R. prowazeki*, *R. ruminantium*, *R. (Deimacentozeus) rickettsia*, lead, in the host animal an exclusively, or preponderingly, intracellular life and form juxtanuclear accumulations which remind one of the Chlamydozoa of Prowazek" (Handb d Path Microorganismen 8: 1347, 1930). In tsutsugamushi disease, the rickettsia found in smear preparations from experimental animals are chiefly in the endothelium and histiocytes. On the contrary, Prowazek's chlamydozoa, the so called trachoma bodies, are found principally in the epithelial cells. Both, however, are very similar in morphology and in staining.

My method for staining trachoma bodies in sections is directly applicable to rickettsia in sections. My method was first published in 1910, in the Acta Societ Ophthal Japon (14 April). By it, Gram negative bacteria as for example, the gonococcus are readily stained in sections. Dr. Y. Onisi, in my clinic (Klin Monatsbl f Augenh 96: June) used my method for the staining of trachoma bodies. Herewith is my method, which is applicable for staining rickettsia in sections.

1. The most suitable fixation solutions for pieces of tissue for my staining method are

- (a) Schnaudinn's sublimate alcohol

- (b) Formalin solution

Commercial formalin

1

Distilled water

9

2. Celloidin imbedding was used in my earlier work, but paraffin is rather better. Sections must not be more than 5 micra thick.

3. The staining solution consists of:

Kresylechtviolet (Grübler)	0.5
Glycerin	10.0
5 per cent carbolic acid	90.0
Distilled water	100.0

This staining solution is for sections, which are thoroughly fixed in sublimate alcohol. If, on the contrary, the sections are fixed in formalin solution, a concentrated staining solution is necessary.

Kresylechtviolet (Grübler)	0.5
Glycerin	10.0
5 per cent carbolic acid	90.0

The stain for these solutions is used as follows: the paraffin sections are laid, section side down, on the bottom of a flat dish. The ends of the slide are raised slightly, so that the sections do not quite touch the bottom of the dish. Then just sufficient staining solution is added to cover the section. This manipulation is designed to minimize precipitation of the stain on the section. The staining time is usually from ten to twenty minutes, at room temperature. More than twenty minutes does no harm. Then the sections are washed, first with distilled water, then differentiated with water acidulated with acetic acid. The acidulated water contains 5 drops acetic acid to 100 c.c. The excess of stain is thus removed from the section. For differentiation it usually suffices to leave the sections one minute in the acidulated water. Then the sections are again well washed to remove the acid. Then the water is absorbed from the stained sections with blotting paper, and they are air dried. Anilin-xytol (1:1) is poured on the sections to remove the last traces of water, and to clear them. Finally the anilin is removed with pure xytol and the sections are mounted in neutral balsam. The manipulations may be epitomized as follows:

1. Sections are imbedded in paraffin.
2. Kresylechtviolet solution, 10 to 20 minutes.
3. Wash with distilled water.
4. Treat with acidulated water.
5. Wash thoroughly with distilled water.
6. Dry sections.
7. Anilin-xytol.
8. Xytol.
9. Mount in neutral canada balsam.

By this procedure, rickettsia, in and out of the tissues, are detectable with strong magnification. The rickettsia appear mostly as tiny spherules, arranged occasionally as diplo- or streptococci. Also, one sees a few elongated rods, or dumbbell-shaped rickettsia. These elongated forms of rickettsia have deeply colored poles, with thinner central parts, but brightly colored.

Rickettsia appear to have intimate relation with the cells in the tissues; particularly one notices them in the substance of endothelial cells and histiocytes. In the parenchyma cells of experimental animals, as liver cells, of a

mouse, I have noticed rickettsia intracellularly Obviously, they are also seen extracellularly, here and there.

The staining method, described above, is very simple, compared with the Giemsa method, and it can be employed for many purposes by anyone. By

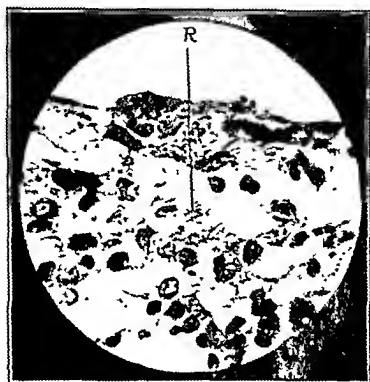


Fig 1.—Peritoneum of mouse In the center is a group of rickettsia (R) $\times 600$

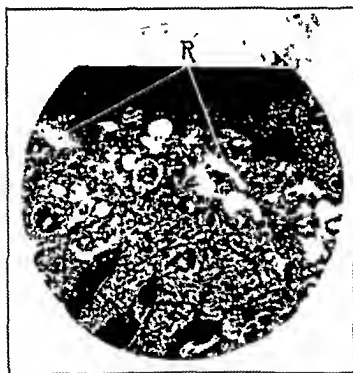


Fig. 2.—Superficial section of the liver of a mouse Rickettsia (R), intra- and extracellular, are readily seen $\times 600$

this procedure of staining sections, one may not only demonstrate rickettsia, but also one has the advantage of detecting, simultaneously, tissue changes as well. It is worthy of note that with my stain, the Kresylechtviolett has a metachromatic property The mast cell granules are colored red brown. They are differentiated readily from rickettsia microscopically in that the latter are always stained a more or less dark violet Also, in size, mast cell

granules and rickettsia are different. The former are somewhat larger than the granule-like rickettsia. I believe that on that account, my staining method for sections, with Kresylechtviolet is very simple, and will prove to be very useful for the study of rickettsia.

The figures show (Fig. 1) rickettsia in the peritoneum, and (Fig. 2) in the liver of a mouse, which were obtained from serial passage of tsutsugamushi disease, sent me by Prof. Dr. N. Ogata, Biological Institute of China Medical College.

A BODY TEMPERATURE REGULATING APPARATUS AND AN IMPROVED AXOGRAPH FOR ANIMAL EXPERIMENTS*

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TEMPERATURE REGULATOR

IN PERFORMING prolonged animal experiments under general anesthesia, the body temperature usually falls until such abnormal conditions prevail as to diminish the value of the results. To combat this, specially designed operating tables have been built with warming devices incorporated in them, or heating units have been put on the artificial respiration machines, when such are being used. None of these devices regulates the body temperature at any given temperature, but simply supplies extra heat. A more suitable method would be to fix the body temperature at the desired level by means of an automatically controlled device, in the same way as is done with water-baths, incubators, etc. I have, therefore, devised a simple electric thermostat, suitable for insertion into the rectum, which controls the heating unit in accordance with the changes in body temperature.

In Fig. 1 is shown a schematic drawing of the thermoregulator used. It is blown from Pyrex glass tubing of about 9 mm. external diameter, with walls of 1 mm. thickness. The general shape is that of an "L," with the bottom or horizontal portion bent slightly upward at an angle of about 85° from the vertical. The horizontal part is about 65 mm. long over all, and is closed off completely from the vertical portion, except for a small hole, 1.5 mm. in diameter, located at the lowermost point of lumen.

A much greater rise in the mercury than could be secured by its expansion alone is obtained by putting into the regulator a few drops of a liquid which boils at about the temperature sought. Its expansion in going from the liquid to gaseous state raises the mercury over a centimeter for each degree centigrade, in spite of the unusual width of the upright tube. Ethyl ether is a satisfactory liquid for temperatures around 37° C., but a somewhat more uniform rise at body temperatures is secured from a mixture of equal parts of the ether and carbon bisulfide.

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Filling and setting the instrument can be completed in very few minutes. The regulator is first filled almost to the top with mercury, then two or three drops of the expansion liquid are dropped on top of the mercury, the finger being placed over the end, taking care to trap a small bubble of air, and the whole is inverted. The expansion liquid and air rise, and by gentle tapping are made to pass through the small hole into the horizontal part. Then almost all of the mercury is poured out of the vertical portion, and it is restored to its upright position. The expansion liquid and air are thus trapped in the horizontal arm, and, as the liquid expands, it pushes the mercury up the ver-

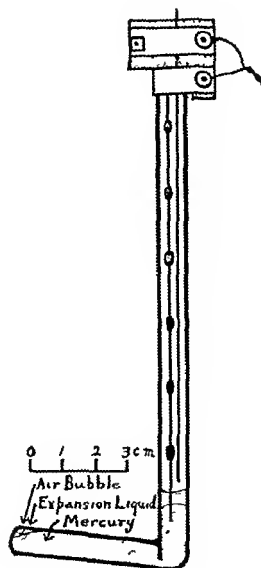


Fig. 1.—Diagrammatic cross section of rectal thermostat

tical arm. To set for temperature the apparatus is immersed in a beaker of water about 1°C hotter than the final temperature desired. The liquid expands as a gas, and, if too much has been put in, the mercury is completely blown out of the horizontal arm, allowing the excess gas to escape. The water is then allowed to cool to the desired temperature, during which process the mercury reenters the horizontal limb as the gas condenses. The adjustable contact rod is then pushed down to the surface of the mercury and clamped in that position. The contact rods are insulated from each other by glass beads threaded on the fixed rod. A drop of methyl salicylate put on top of the mercury prevents oxidation during operation of the thermostat.

In actual use, the horizontal limb is inserted into the rectum, and the thermostat held in place in the upright position by a clamp on a stand. The contacts of the thermostat control through a relay the heating pad or whatever source of external heat is being used. A very satisfactory and inexpensive relay for this purpose is the Precision Temperature Regulator,* which is so constructed that it requires only plugging in to the electric light socket to supply the current for both the heater and the thermostat. No secondary source of low voltage for the thermostat is required. As a heater, we ordinarily use any of the market brands of electrical heating pads, covered with rubber cloth to keep them dry, or the all-rubber one made by the Seamless Rubber Company. When working with small furry animals, such as cats, where heat transference from the pad to the animal is very slow, a pad of low heat capacity is best to prevent too much overswing in the regulation of the temperature. Clipping the hair off the back also increases the speed of heat transfer and makes the regulation more sensitive. Although this apparatus has been used only in animal experiments, there can be little doubt that the principles involved would be equally applicable to the maintenance of body temperature of patients under conditions of shock, collapse, prolonged poisoning, etc., where this is a major factor in promoting recovery of the patient, and ordinarily requires continuous nursing attention.

AN IMPROVED AXOGRAPH

In making kymographic records, particularly of blood pressure, where the amount of rise or fall is important, it is a great convenience to have automatically drawn on the record a scale from which the magnitude of the changes can be read off at a glance. An instrument for this purpose was described and named an axograph by Gautrelet¹ some years ago. Gautrelet's apparatus had the disadvantage that it was mounted rigidly on a clamp and spring bar, so that any irregularities in the paper, or lack of exact parallelism in the support rod, prevented contact over the whole width of the paper. It did not prove satisfactory for general use. The axograph pictured in Fig. 2 is simpler to make and free from this disadvantage.

As shown in the schematic cross-section, the supporting rod fits into the sleeve of the axograph. A round pin passes through this sleeve, engaging in a slot in the support rod, thus fastening it in, but permitting complete revolution of the axograph on the rod. In addition, the supporting rod is placed nearer to one end of the axograph, so that when the rod is held horizontally the face of the axograph hangs in the vertical position by the force of gravity and parallels the surface of the paper. The revolving bar of the axograph is very simply constructed by using a gear bar of the desired length, and about 15 mm. in diameter, and cutting it out on a lathe to leave circles of teeth 5 mm. apart along its length. The appearance of this in cross-section at the level of one row of the teeth is shown in Fig. 2. When the axograph is held in contact with the smoked paper, the bar revolves, drawing a series of dotted lines 5 mm. apart on the paper, which correspond to 10 mm. of blood

*This regulator and the thermostat can be obtained from the Eastern Engineering Company, New Haven, Conn.

pressure when the ordinary type mercury manometer is used. In having the bottom line of dots at the zero level the blood pressure can be read off very readily, as shown on the record in Fig. 2. The revolving bar can be made whatever length is required for the width of the kymograph paper customarily used, the one pictured being used for 10 inch paper. The advantage of this axograph over Gautrelet's is in the swivel joint in the supporting rod, which permits the revolving bar to swing out of its vertical plane and remain in contact with the entire width of the paper, in spite of irregularities on the surface or wrinkles that are so frequent on long paper kymographs.

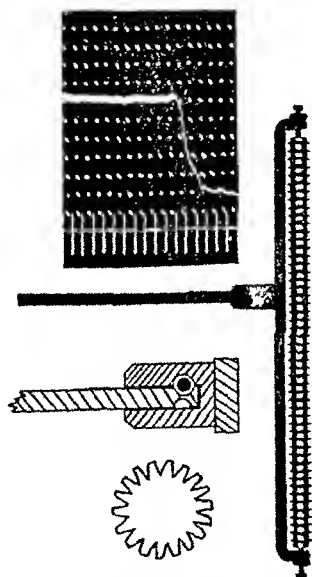


Fig. 2.—Photograph of axograph, cross section drawings of revolving bar and central pivot construction (not to scale) and typical blood pressure tracing showing the dotted lines drawn on the record by the axograph.

In actual use, the axograph is mounted on a long paper kymograph, clamped to an upright rod at the far side of the apparatus. It is swung into contact with the paper between the drums, with sufficient pressure to give firm contact. The drum is then quickly revolved through a complete turn of the paper, dotting in the axograph record as it revolves. Then the instrument may be swung away from the kymograph so as to be out of the way when the paper is taken off or fresh paper put on.

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In actual use, the horizontal limb is inserted into the rectum, and the thermostat held in place in the upright position by a clamp on a stand. The contacts of the thermostat control through a relay the heating pad or whatever source of external heat is being used. A very satisfactory and inexpensive relay for this purpose is the Precision Temperature Regulator,* which is so constructed that it requires only plugging in to the electric light socket to supply the current for both the heater and the thermostat. No secondary source of low voltage for the thermostat is required. As a heater, we ordinarily use any of the market brands of electrical heating pads, covered with rubber cloth to keep them dry, or the all-rubber one made by the Seamless Rubber Company. When working with small furry animals, such as cats, where heat transference from the pad to the animal is very slow, a pad of low heat capacity is best to prevent too much overswing in the regulation of the temperature. Clipping the hair off the back also increases the speed of heat transfer and makes the regulation more sensitive. Although this apparatus has been used only in animal experiments, there can be little doubt that the principles involved would be equally applicable to the maintenance of body temperature of patients under conditions of shock, collapse, prolonged poisoning, etc., where this is a major factor in promoting recovery of the patient, and ordinarily requires continuous nursing attention.

AN IMPROVED AXOGRAPH

In making kymographic records, particularly of blood pressure, where the amount of rise or fall is important, it is a great convenience to have automatically drawn on the record a scale from which the magnitude of the changes can be read off at a glance. An instrument for this purpose was described and named an axograph by Gautrelet¹ some years ago. Gautrelet's apparatus had the disadvantage that it was mounted rigidly on a clamp and spring bar, so that any irregularities in the paper, or lack of exact parallelism in the support rod, prevented contact over the whole width of the paper. It did not prove satisfactory for general use. The axograph pictured in Fig. 2 is simpler to make and free from this disadvantage.

As shown in the schematic cross-section, the supporting rod fits into the sleeve of the axograph. A round pin passes through this sleeve, engaging in a slot in the support rod, thus fastening it in, but permitting complete revolution of the axograph on the rod. In addition, the supporting rod is placed nearer to one end of the axograph, so that when the rod is held horizontally the face of the axograph hangs in the vertical position by the force of gravity and parallels the surface of the paper. The revolving bar of the axograph is very simply constructed by using a gear bar of the desired length, and about 15 mm. in diameter, and cutting it out on a lathe to leave circles of teeth 5 mm. apart along its length. The appearance of this in cross-section at the level of one row of the teeth is shown in Fig. 2. When the axograph is held in contact with the smoked paper, the bar revolves, drawing a series of dotted lines 5 mm. apart on the paper, which correspond to 10 mm. of blood

*This regulator and the thermostat can be obtained from the Eastern Engineering Company, New Haven, Conn.

of citrate solution, the cell volumes in the five portions were adjusted to 25, 29, 35, 40, and 45 per cent, and the citrate concentration in the plasma of each sample was brought to the appropriate amount. Adjustment of the citrate concentration in the plasma is necessary since when in routine work blood is drawn from patients, the citrate concentration in the plasma is influenced by the per cent cell volume of the blood. The well mixed samples were placed in flat bottomed sedimentation tubes of 4 mm bore, which gave a blood column 100 mm high. After one hour the observed sedimentation was recorded in

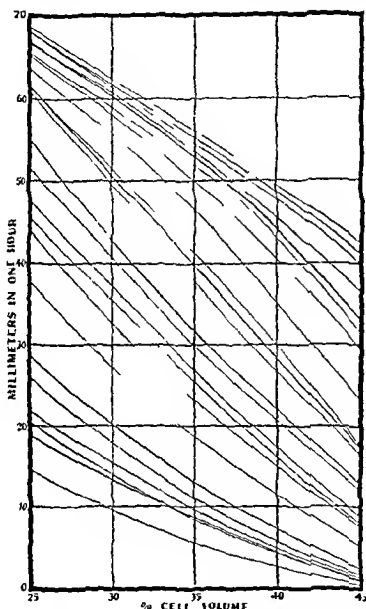


Fig 1—Influence of *in vitro* change in cell volume upon the sedimentation rate

millimeters, the samples were then centrifuged thirty minutes at 3 000 r p m and the percentage volume of packed red cells read off directly. The results thus obtained are given in Fig 1. Three blood samples gave curves so closely alike that they are represented by only one line in the figure, the same is true for two other pairs of curves. No attempt was made to specially select the patients since it is clear from the results of Gram³ and Rouke and Einstene⁴ that the *in vitro* influence of cell volume upon the sedimentation rate is a consistent one, which is not related to the particular infection. By averaging the results of Fig 1, the final correction chart given in Fig 2 is obtained.

DETAILS OF TECHNIQUE

In routine use of this method, 2 c.c. of blood is added to 0.35 c.c. of 3.8 per cent citrate. Accurate measurement is essential, especially of the citrate, since the same sample is used both for a cell volume estimation and for the sedimentation test. Using a 2 c.c. long precision type of syringe and the technique indicated, we find that the syringe transfers 0.05 ± 0.01 c.c. of citrate to the blood sample;* the remainder of the citrate, 0.30 c.c., is placed in the small tube which receives the blood. The sedimentation tubes are 11 c.c. long and of 4 mm. bore; they may be graduated in millimeters to give a blood column 100 mm. high—the Wintrobe hematocrit tube⁶ is satisfactory, or the tubes may have a single graduation mark 100 mm. above the floor of the tube, readings being taken by a millimeter scale held against the tube.

METHOD

Use a 2 c.c. precision type syringe, dry and sterile, and a 1 inch 20-gauge needle. Draw sterile 3.8 per cent citrate into the syringe, and run the piston up and down, so as to wet all the inside of the syringe. Then drive the piston completely in while the needle is pointed upward, so that all air bubbles are removed and the needle is left full of citrate.

Enter a vein and draw blood accurately to the 2 c.c. mark. Withdraw from vein, quickly detach and discard the needle, invert the syringe over a small tube containing the additional volume (0.30 c.c.) of 3.8 per cent sodium citrate, and press in the piston so that the blood flows vertically downward into the citrate in the tube. Do not expel the blood against the side of the tube. Immediately close the tube with a clean cork and invert and rotate it 10 times. The tube containing the citrate must be uncorked and conveniently placed before commencing to draw the blood, as there should be no delay in the operation. The sample should not be used for fifteen minutes, so that it may reach room temperature. Within five hours from the time of drawing the blood, it is well mixed by gently inverting and rotating the tube for at least ten times; it is well to hold the tube by the corked end, to avoid warming the sample by the heat of the hand; with a capillary pipette fill the sedimentation tube to the zero mark, taking care to avoid air bubbles. After standing one hour in a perfectly vertical position, the degree of sedimentation is read off in millimeters. The tube is then centrifuged 30 minutes at 3,000 r.p.m., and the percentage volume of packed red cells is read off directly from a millimeter scale.

From the graph of correction curves the observed sedimentation rate is corrected to standard cell volume, i.e., to the 37 per cent cell volume line of Fig. 2. This cell volume, 37 per cent, is chosen as it corresponds to 42.5 per cent cell volume in the undiluted blood, which, using Gram's figures, would be the mean of the average cell volumes for both men and women. To use the graph in routine work that point upon the graph is located which corresponds to both the cell

*The amount of citrate transferred to the blood is found by blank trials with this technique, using N/10 NaOH in place of citrate, and distilled water in place of blood. The volume of NaOH carried over into the water is found by titration with acid.

volume and the observed sedimentation rate of the blood sample; the curve upon which this point lies, or the interpolated curve if it lies between two curves, is then followed to the standard cell volume (37 per cent) line, where the position of the curve gives the sedimentation rate for standard cell volume. For example, if the observed sedimentation is 20 mm and the cell volume 33 per cent, find the figure 20 on the left hand scale of Fig 2, and follow the horizontal line opposite this figure until vertically over the figure 33 of the bottom line. The point thus located is found to lie on one of the curves; following this curve down to the

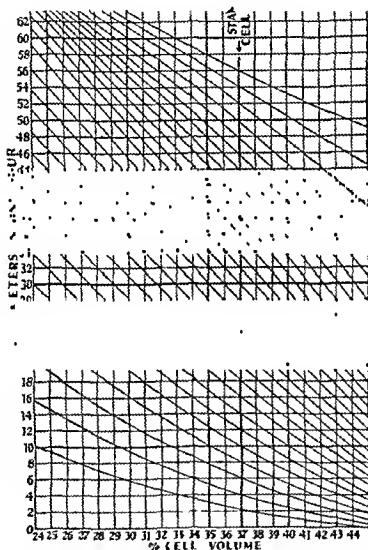


Fig. 2—Chart for correcting observed sedimentation rates to standard cell volume (37 per cent). The cell volume figures refer to the citrated blood, 85 vol. blood to 15 vol. of 3.8 per cent citrate, used in the test

standard cell volume line, the two intersect at a point opposite figure 14 of the left hand scale. This figure gives the sedimentation rate, corrected to standard cell volume.

NORMAL STANDARDS

For corrected sedimentation rates obtained by the method described, the following may be taken as the standards for both men and women.

Normal	1 to 7 mm.
Borderline	8 to 10 mm.
Pathologic	11 mm. and over

Correction for cell volume has been advocated by several authors^{3, 4} in the belief that it eliminates inaccuracies resulting from variations in the red cell content of the blood. However, it should be kept in mind that all methods of correcting for cell volume are based on in vitro findings, when the ratio of red cells to plasma is artificially altered. In practice, there may be other factors involved,² so that correction for cell volume may introduce greater errors than it eliminates. It was to investigate this point that the foregoing method was devised; comparisons of clinical and x-ray data to the sedimentation rates were made by Dr. D. W. Crombie. The results obtained indicate that correction for cell volume is in general of advantage for blood containing over 45 per cent cell volume, i.e., 39.2 per cent cell volume in the citrated sample, especially when the sedimentation rate is relatively low. On the other hand, correction for cell volume is in general uncalled for or undesirable in the case of blood having normal or subnormal cell volume.

Acknowledgment.—The authors are indebted to Dr. D. W. Crombie, Medical Superintendent, and to Dr. P. M. Andrus, Director of the Mara Laboratories at the Queen Alexandra Sanatorium, who suggested and made possible this work. The technical assistance of Mr. R. Comrie is also acknowledged.

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A DIRECT READING NUL POINT AMPLIFIER FOR MEASURING HYDROGEN ION CONCENTRATIONS AND OXIDATION- REDUCTION POTENTIALS*

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SKOW and Wynd¹ have published a description of a single tube amplifier for use in the determination of glass electrode potentials, which is characterized by operating at a low filament temperature, low plate voltage, and with a grid bias equal to the free grid potential. The present paper describes modifications of the apparatus whereby the measuring part of the circuit can be so adjusted as to cause the apparent potential value to equal the pH of the unknown solution, irrespective of the temperature.

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Fig 1 shows that the amplifying part of the circuit is similar to that previously described. A potentiometer (R8, R9, B5) has been inserted in the measuring part of the circuit to cancel the calibration constant of the system.

The battery (BA) of the measuring potentiometer applies its potential to the calibrated resistance through appropriate resistances (R10, R11), so that the voltage drop between successive contact terminals of the potentiometer is no longer 0.100 volt, as usual, but is equal to the theoretical change in millivolts per pH unit. The authors have found it convenient to distribute R11 between fixed and variable resistances so as to allow R10 to carry only that part of the load needed to correct the total resistance for the effects of temperature in the pH millivolt ratio.

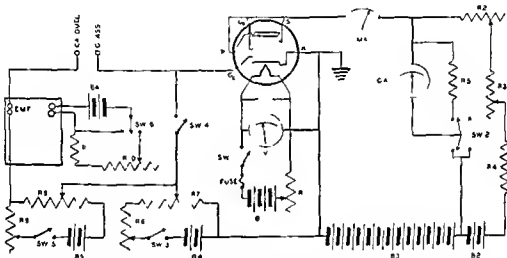


Fig 1—The theoretical wiring diagram of the amplifier. Type 89 vacuum tube 0.1 D.C. milliammeter 0.6 D.C. voltmeter 1000 ohms per volt Cal. Leeds and Northrup 2320 d. galvanometer R1 20 ohms R2 100 ohms R3 10,000 ohms R4 1,000 ohms R5 100 ohms R6 400 ohms R7 15,000 ohms R8 2,000 ohms R9 20,000 ohms Sw 1 0.6 and reversing f.p.d. Federal antiparallel switch Sw 4 s.p.d. Federal antiparallel switch B1 6 volt storage battery B2 B3 B4 3 to 4.5 volts B batteries B5 19.5 volts from radio B batteries BA potentiometer battery

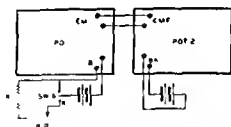


Fig 2—Method of calibrating the temperature correction dial

The calibration of R10 is accomplished empirically by balancing two potentiometers against each other as shown in Fig 2. With Switch 6 at position A, Potentiometer 1 is standardized as usual by its standard cell. Potentiometer 2 is then standardized with the same standard cell. Switch 6 is then thrown to position B. Potentiometer 1 is then set to read 1.000 volt, and Potentiometer 2 is set to read 0.5733 volt. Adjust R10 until balance is obtained. The range of Potentiometer 1 now varies numerically parallel with pH at a temperature of 16° C. An appropriate mark is made on the instrument panel to record this position. Potentiometer 2 is then set to read 0.5773 (voltage change for each ten pH units at a temperature of 18° C) and the process described above re-

peated. The necessary values for temperature corrections from 16° C. to 42° C. may be obtained from Table I.

TABLE I
THE CHANGES IN VOLTAGE (D) PER PH UNIT AT DIFFERENT TEMPERATURES

TEMP. ° C.	VOLTAGE CHANGES PER PH UNIT	TEMP. ° C.	VOLTAGE CHANGES PER PH UNIT
12	0.0565	28	0.0597
14	0.0569	30	0.0601
16	0.0573	32	0.0605
18	0.0577	34	0.0609
20	0.0581	36	0.0613
22	0.0585	38	0.0617
24	0.0589	40	0.0621
26	0.0593	42	0.0625

The resistance values of R10 and R11 will depend on the type of measuring potentiometer (Potentiometer 1) used. For this reason a preliminary determination of the total ohms resistance needed should be made, using a resistance box in place of R10 and R11. Providing an instrument such as Leeds and Northrup Potentiometer 7655 is used, R10 should be approximately 125 ohms, while R11 can be conveniently distributed between a 350 ohm fixed resistance and a 0 to 100 ohm variable resistance. For a Type K potentiometer these values should be 30, 50, and 0 to 50 respectively.

It is obvious that the measuring potentiometer must be standardized with Switch 6 at position A, and that in this position of the switch the E. M. F. readings on unknown solutions are expressed in millivolts. This switch enables the operator to instantly convert the apparatus from a direct reading pH meter to a standard potentiometer for the determination of pH by calculations or of oxidation-reduction potentials.

Assuming that the apparatus is wired according to Fig. 3, the operation is as follows:

1. Depress Switch 1. This closes the circuits indicated in Fig. 1 by Switches 1 and 3. When tube 89 is used, adjust R1 until the milliammeter indicates a plate current of 0.6 to 0.8 milliampere. If tube 38 is used, adjust until the plate current is 0.3 to 0.4. When R1 is set, it needs no further adjustment for many months.

2. When the plate current has become constant (three to five minutes), depress Switch 2. This completes the galvanometer circuit so that the major portion of the current is shunted past the galvanometer, thus protecting this instrument until the circuit is balanced. Balance with R2 and R3 until the galvanometer reads zero, or approximately zero. Slight instability of the galvanometer may be observed at this stage, but this in no way affects the final accuracy of the readings. The balancing of the galvanometer in this condition of low sensitivity is necessary only during the process of determining the original zero point. It is not necessary during the determination of successive pH readings.

3 Depress Switch 4 and balance by adjusting R6 and R7 until the galvanometer needle is stabilized. This can be at any point on the galvanometer dial, but for convenience should be at the zero position. Open Switch 4.

4 Raise Switch 2 to the upper position (position of maximum sensitivity of the galvanometer) and rebalance as in Step 3. Switch 2 remains in the position of maximum sensitivity during the determination of pH readings.

5 Raise Switch 6 to the upper position, and standardize the measuring potentiometer. If readings are to be made in terms of volts, Switch 6 remains in this position. The unknown solution in which the glass or platinum electrode is inserted, is now bridged to the calomel cell. For this purpose a permanent bridge, filled with saturated potassium chloride solution, consisting of a pyrex "U" tube in the ends of which have been sealed porous porcelain plugs, can

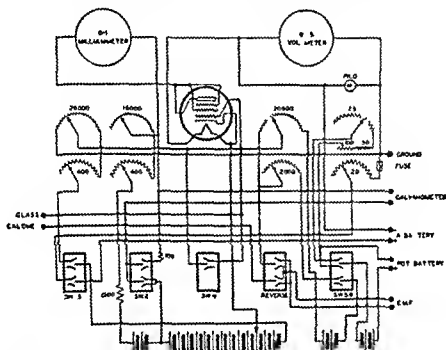


Fig 3—Actual hook up of the amplifier. The terminals indicated by glass and calomel are attached to the jack connections of the electrode set up illustrated in Fig 6. The terminals indicated as ground, galvanometer, battery, pot battery, and E.M.F. are led through the metal cabinet by porcelain insulators 2 inches long as indicated in Fig 4. The parts of the circuit thus exposed are sufficiently insensitive to shielding to enable the indicated connections to be made externally. The ground terminal is soldered to a water pipe. The galvanometer terminals are attached to the indicated terminals of the galvanometer as indicated in Fig 5. The 6 battery terminals are attached to a 6 volt storage battery. The pot battery terminals are attached to the battery terminals of the measuring potentiometer; the battery itself is within the cabinet. The E.M.F. terminals are attached to the corresponding terminals of the measuring potentiometer as indicated in Fig 5.

conveniently be used. The measuring potentiometer is adjusted until the galvanometer needle shows no deflection upon depressing Switch 4. This again can be at any point on the galvanometer scale.

6 If readings are desired directly in pH units, first standardize the measuring potentiometer as in Step 5 with Switch 6 in the upper position. Then depress Switch 6 and bridge a solution of known pH (as for instance, M/20 potassium phthalate buffer—pH 3.97) to the calomel cell. Set the temperature correction dial R10 to the position corresponding to the temperature of the known buffer, and balance with R8 and R9 until the galvanometer needle shows no deflection on closing Switch 4. Unknown solutions may now be sub

stituted for the known buffer and their hydrogen ion concentration read directly. For the greatest accuracy, the pH of the known standardizing buffer should approximate that of the unknown solution. If well insulated electrodes are used and the tube is thoroughly warmed, readings accurate to ± 0.003 pH unit can be made. In the determination of successive pH readings, it is only necessary to rinse the glass electrodes, substitute another solution, and balance with the measuring potentiometer until the galvanometer needle shows no deflection when Switch 4 is tapped downward. Hence, successive readings can be made in a few seconds.

When the pH is being determined by calculation from the readings in millivolts, the readings of a standard buffer must first be obtained. The following formulas are then used. Switch R, in Fig. 4, reverses the terminals of the measuring potentiometer.

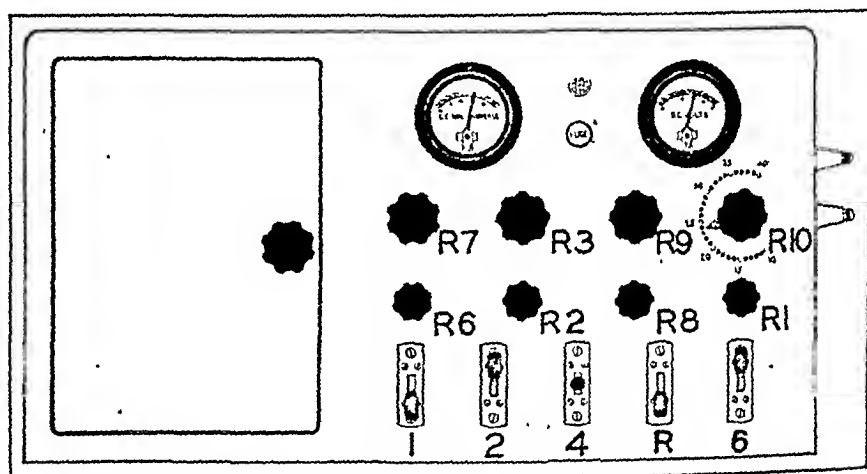


Fig. 4.—Diagram of front of box showing position of the switches and dials. These positions correspond to those shown in Fig. 3.

1. If the observed potential is greater than that of the standard buffer (higher pH, the reversing Switch R will be down).

$$\text{Unknown pH} = C + \frac{(A - B)}{D}$$

2. If the observed potential is less than that of the standard buffer (lower pH, but Switch R down),

$$\text{Unknown pH} = C - \frac{(B - A)}{D}$$

3. When reversing Switch R is up (very low pH values):

$$\text{Unknown pH} = C - \frac{(A + B)}{D}$$

where A = potential of the unknown; B = potential of the standard buffer; C = pH of the standard buffer, and D = voltage change per pH unit at the temperature of the unknown solution (see Table I).

Switch 6 in Fig 4 is so wired that when it is in the upper position, the circuit indicated by Switch 5 in Fig 1 is broken. This causes the potentiometer (R9, B5) to function only when pH readings are being made directly.

Since a galvanometer is needed for the preliminary standardization of the measuring potentiometer, it follows that two galvanometers are needed for the complete apparatus, provided a potentiometer with a built in galvanometer is used. A simple modification of the wiring of the potentiometer will permit the built-in galvanometer to be used first to standardize the measuring potentiometer and then by throwing a switch to function in the plate circuit of the amplifier. Fig 5 shows the changes necessary for a Leeds and Northrup potentiometer Type 7655.

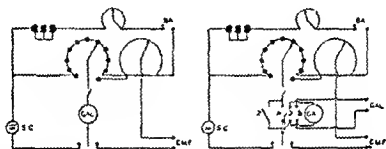


Fig 5.—Wiring changes of built in galvanometer type potentiometer to obviate the use of a second galvanometer. The diagram to the left indicates the original potentiometer wiring the figure on the right indicates the modifications. The switch indicated may be a double pole double throw Federal antistatic switch mounted at any convenient place in the potentiometer box.

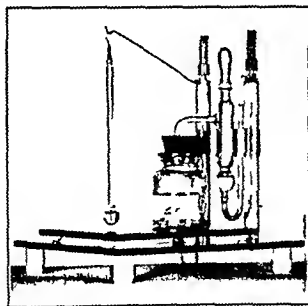


Fig 6.—Electrode set up. The jack connections at the ends of the vertical insulators are attached to the corresponding terminals indicated in Fig 3. The electrode set up is of course shielded within the grounded metal cabinet.

The electrode set up is shown in Fig 6. By means of the double shank glass electrode,² errors due to electrical leakage across the electrode surfaces are reduced to an insignificant magnitude. The side arm of the calomel reference electrode dips into a saturated potassium chloride solution, contained in a bottle fitted with a two hole rubber stopper. The upper portion of the bottle is smeared with lanolin to prevent creeping of the potassium chloride. The glass areas of the permanent bridge should likewise be covered with a very thin layer of lanolin, so that the porcelain tips can be kept in saturated potassium chloride solution when the apparatus is not in use.

The very great resistances of the type of glass electrodes used necessitate careful shielding and grounding of the apparatus. The metal cabinet shown in Fig. 4, connected to the circuit as shown in Fig. 3, is grounded.

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A METHOD FOR UTILIZING ILLUMINATING GAS IN THE BROWN, FILDES AND MCINTOSH OR OTHER ANAEROBE JARS OF THE LAIDLAW PRINCIPLE*

JOHN H. BREWER, M.A., AND J. HOWARD BROWN, PH.D., BALTIMORE, MD.

THE most satisfactory method for the cultivation of anaerobes makes use of some type of jar in which the oxygen is completely burned out by means of a catalyst, originally employed by Laidlaw (1915). This method eliminates the trace of oxygen, which is always present in evacuation and replacement systems, and which seriously interferes with the cultivation of the most strict anaerobes, such as *Cl. novyi*. The principal objections to the use of these jars have been the initial cost of equipment and the earlier explosions which occurred with improperly designed and poorly constructed jars. The danger has been eliminated in more recently designed equipment.

Hall (1929) reviews the success and failure of several workers in using illuminating gas for replacement after evacuation, although he makes no mention of the use of this gas in catalyzer systems. We have tried over a period of three years to use gas from the laboratory jet in the Fildes and McIntosh (1921) and Brown (1921) jars, but without complete success until this past year. It was supposed that the gas had some toxic effect as has been reported by Kladakis (1890), Ludwig (1918), and Frankland (1889). Kladakis is the only one of these who used an anaerobic organism. His technique of bubbling the gas through the small amount of medium for thirty minutes would saturate it with the carbon dioxide present, so that the pH would not be in the proper range for growth to occur. Ludwig and Frankland used aerobic or facultative organisms whose growth would naturally be inhibited to some degree by anaerobic conditions. Their results were largely due to a lack of oxygen, and not to toxic action of the gas.

Wurtz and Fonreux (1889) used illuminating gas for freeing the medium of air, and for keeping the air out of the test tube until they made an inoculation under an oil seal; they did not actually cultivate the organism in the presence of the gas.

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Bullock (1900) and Wilson (1917) employed illuminating gas to flush the air out of special containers, then used pyrogallie acid and alkali to remove the remaining oxygen. The large amount of alkali used (109 gm to 145 cc of water) was sufficient to absorb much of the gas so that the pH would not be lowered. Wilson reported excellent growth of *Cl sporogenes*, *Cl septicus*, *Cl histolyticum*, *Cl oedematiens*, and *Cl tetani*. In his system there was unquestionably some gas present, but the toxic effect of the carbon dioxide was certainly eliminated by the alkali present.

Holker's (1920) success in using coal gas is readily explained in the earlier part of his paper. "At the outset, it is to be pointed out that the bacteria are not grown in an atmosphere of coal gas nor were they grown in an atmosphere of hydrogen. . . The coal gas or the hydrogen, as the case may be, serves merely to wash the oxygen from the vessel. When the vessel is finally closed off in vacuum, both the oxygen and coal gas remaining are very small in amount."

Brown, in describing the use of his jar suggests that the hydrogen be allowed to diffuse into the jar at about five pounds' pressure. The gas pressure at a laboratory jet is usually about one fortieth this pressure. Because of this low pressure, not enough of the gas will flow into the jar to start combustion. If the jar is attached to an ordinary inexpensive water vacuum pump for about one minute and then attached to the gas jet enough gas will be drawn in to start combustion. A manometer need not be used but if convenient may be employed and the jar evacuated to about 50 cm of mercury. This is not to be confused with an evacuation replacement method, it is only to get sufficient gas into the jar, under low pressure, to initiate combustion. The jar is allowed to remain attached to the jet after the electric current is turned on for about thirty to forty five minutes, then the electric and gas currents turned off and the jar sealed.

Since the gases found in municipal supplies are usually ethane, methane, hydrogen, carbon monoxide, and illuminants, all of which burn to carbon dioxide and water, there will be formed an excess of carbon dioxide under a slight pressure which will lower the pH of the media about 0.4. Valley and Rettger (1927) made a very exhaustive study of the effect of carbon dioxide on bacteria. This study includes *Cl sporogenes*, *Cl welchii*, *Cl tetani*, and *Cl putrificum*, and

TABLE I
TYPES OF GAS SUITABLE FOR USE AS DESCRIBED

	NEW YORK	PHILA DELPHIA	BALTI MORE	CINCINNATI*		CHICAGO	ST LOUIS	LOS ANGELES
Illuminants	5.71	5.9	3.9	0.1	1.5	2.1	4.0	
Hydrogen	45.4	37.7	36.1	9.8	18.7	24.6	25.6	
Methane		17.6	24.6	66.0	67.5	55.7	47.5	80.0
Ethane	26.1	1.9		8.0	4.7	6.9	9.2	16.6
Carbon dioxide	2.4	5.2	3.5	2.9	1.4	1.0	1.0	1.0
Carbon monoxide	8.2	16.0	13.0	8.4	3.1	2.9	2.9	
Oxygen	0.9	0.9	1.0	0.2	0.4	0.3	0.7	0.7
Nitrogen	13.3	14.8	17.9	4.6	2.7	6.7	9.1	1.7

*Two types of gas are used in Cincinnati

†Analyses are in per cent by volume

their conclusions are that any harmful action brought about on bacteria by carbon dioxide is due to increased hydrogen ion concentration, although they found that a certain amount of carbon dioxide actually favored the growth of bacteria. To offset this increase in hydrogen ions the media may be buffered,

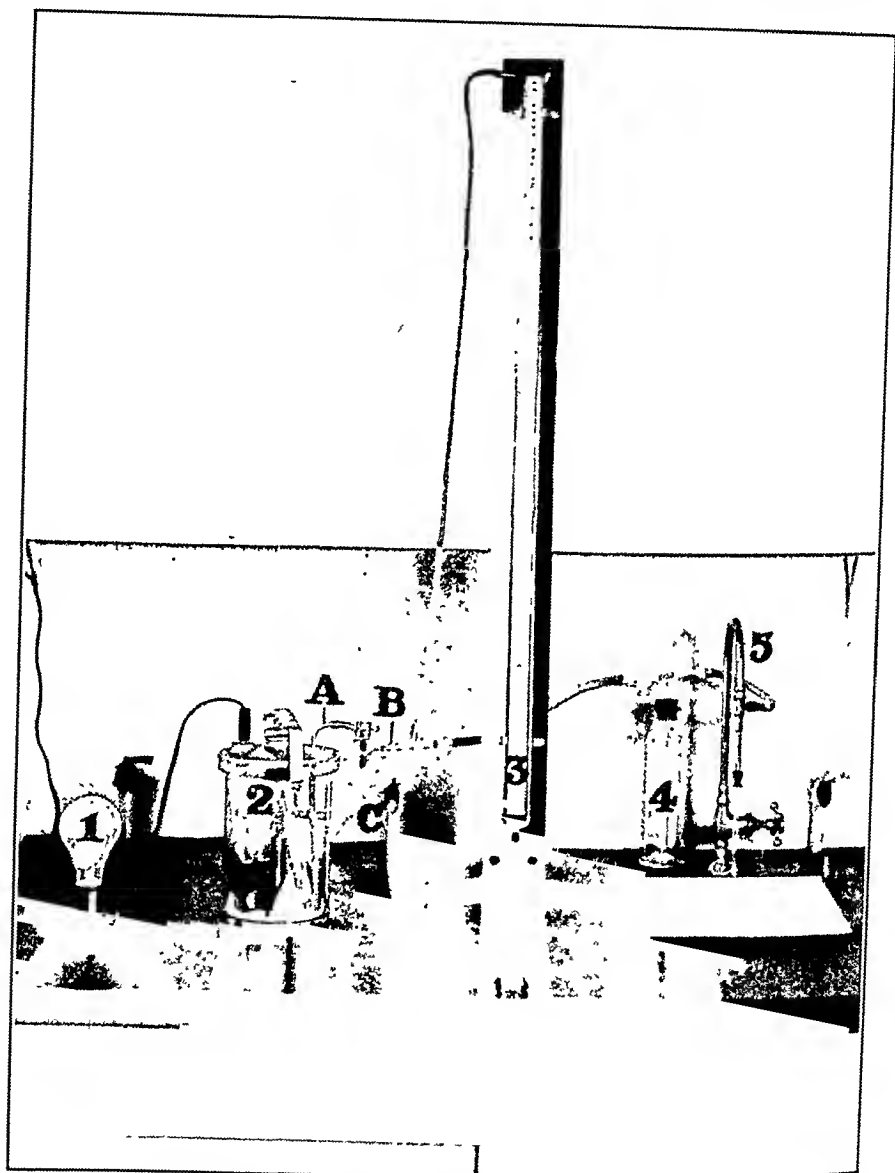


Fig. 1.—1, A 32-candle power carbon filament lamp in series as a resistance unit; 2, improved type Brown jar, manufactured by the Scientific Equipment Co., Philadelphia; 3, manometer connected in the vacuum line by T-tube; 4, water trap; 5, water vacuum pump. A, Outlet to jar. Rubber tube leads to bottom of jar to aid diffusion; B, vacuum connection; C, illuminating gas jet connection (Photograph Courtesy of Hynson, Westcott & Dunning, Baltimore).

but we have found it more convenient to enclose a test tube of soda lime which may be used repeatedly for at least five weeks. The use of soda lime is perhaps more desirable because any medium may be used without the addition of a buffer.

The use of gas from a laboratory jet not only reduces the cost of the set up to about one third its former price, but there is no need for a hydrogen cylinder or generator. Illuminating gas is less explosive and is used at less than one twentieth to one fiftieth of the hydrogen pressure.

In order to determine whether this method would be applicable in other cities where the municipal supply is somewhat different from that in Baltimore, analyses of supplies in various sections of the United States were obtained, samples were secured from some of them and used in the jars. If properly used, good growth should be obtained from any gas where the analysis is similar to those shown in Table I.

This anaerobic system, using illuminating gas, is being employed successfully in several laboratories in Johns Hopkins Hospital and Medical School, and in one commercial laboratory a large number of sterility tests are handled in this way very inexpensively as compared with former methods. A control tube containing *Cl novyi* in 1:100,000 dilution is included in each jar, and good growth in forty eight hours demonstrates the absolute anaerobiosis in the jar.

The equipment shown in Fig. 1 illustrates the usual arrangement the cost of which is less than twenty five dollars. The effort and time (three to five minutes) which is usually required to place the tubes in the jar roll the plasticine seal, partially evacuate and attach the gas, may be lessened if a three way stop cock is attached to the vacuum (B) and gas (C), and then to the jar outlet (A). After closing the jar, it is only necessary to plug in the current (110 volts through a 32 C P. carbon filament lamp in series). When about a 50 cm. vacuum is reached, the stopcock is turned so that the gas will flow into the jar. After thirty to forty five minutes the valve at (A) is closed and the gas and electric currents discontinued.

The use of electrically heated catalyzer systems for the detection of anaerobes in products in ampoules and other preparations has several advantages over many other methods which are satisfactory for the less strict anaerobes, such as *Cl sporogenes*, *Cl welchii*, and *Cl tetani*. One of these advantages is that in testing the sterility of biological products a large number of tubes may be tested at one time, and not only will the anaerobes which have a comparatively high oxygen tolerance be picked up but the most fastidious organisms as well, many of which are entirely overlooked when Smith tubes or similar methods are employed. Another advantage is that absolute anaerobiosis exists from the time the jar is placed in the incubator and is not dependent on slow working catalyst or vegetable tissue systems in which forty eight hours may elapse before an anaerobic state is reached.

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THE WEIGHT CURVE COMPLICATED BY DEATHS*

R. D. TEMPLETON, AND MARY C. PATRAS, CHICAGO, ILL.

IF ONLY the surviving animals are considered in calculating an average weight curve, the death of individual animals frequently gives false implications to the results. For example, the average weight of four animals (Table I) was 175 gm. at a time when one animal was much smaller than the others. The death of this small animal and the dropping of it from the weight curve caused an apparent average gain in weight of 18 gm., when actually all of the surviving animals lost weight. This apparent gain is due to the fact that the dropping of one animal from the total of four is a reduction of 25 per cent in the number of animals, while the withdrawal of 100 gm. (i.e. the weight of the discarded animal) from the total of 700 gm. (i.e. the total weight of the four animals) is a reduction of only 14.2 per cent.

TABLE I

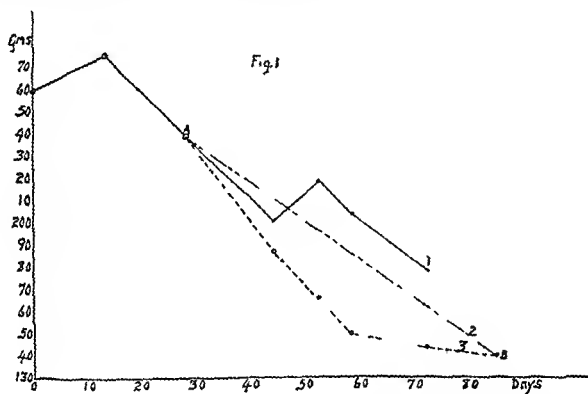
NUMBER OF ANIMALS	WEIGHTS BEFORE DEATHS	NUMBER OF ANIMALS	WEIGHTS AFTER ONE DEATH	CHANGES IN WEIGHT
1	200	1	195	-5
1	200	1	190	-10
1	200	1	195	-5
1	100	0	0	
Totals 4	700	3	580	
Averages	175		193	

To eliminate the objection referred to, an experiment may be considered as a unit consisting of several parts, each part a vital constituent until the ultimate end, the ultimate end of such an experiment being the death of all its parts. If an experiment is considered as a unit, no individual animal can be discarded before the termination of the unit. If the animal is to be considered throughout the course of the experiment, obviously its weight must be considered also.

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The death of an animal, although terminating it as an individual, does not eliminate it from the experiment, and, therefore, its weight at death must be carried as a part of the total weight of the unit. By using the total number of animals in the unit as the divisor and the total weight of the unit as the dividend, a curve is obtained which represents the weight of an average animal and is parallel to the curve representing the total weight of the experimental unit.

The fallacies of the weight curve where only the surviving animals are considered are especially noticeable when dealing with adult animals on a deficient diet. One of our studies in vitamin B insufficiency included 36 adult male albino rats, which prior to the experiment received a stock diet of Fox Chow ad libitum, with bread, meat, and cabbage once per week. From the start of the experiment these animals were given a diet consisting of casein, 18 per cent,



starch, 57 per cent, butter, 15 per cent, baker's yeast, 5 per cent, cod liver oil, 2 per cent, and inorganic salts (Harris), 3 per cent. They were weighed at regular intervals during the course of the experiment.

When only the surviving animals were considered in calculating the average weight of each unit (Fig 1, curve 1), the curve seemed significant for the first forty-four days. During the first thirteen days there was a small gain in the weight, indicative of some vitamin B having been stored in the tissues of the animals while on a good diet. During the next fifteen days a decline was observed in the weight, which indicated a diminution in the stored vitamin B to the extent that an inadequacy for the well being of the animals had developed. During the succeeding sixteen days (twenty-eighth to forty-fourth) 7 animals died, however, the diminution in the weight of the surviving animals was sufficient to also show a marked decrease in the weight curve. Between the forty-fourth and fifty-second day 15 animals died. The discarding of these

animals from the experiment was relatively much greater than dropping the weight of the discarded dead animals from that of those surviving. Thus an apparent gain in weight for the average surviving animal was indicated, even though there was actually a significant loss of weight.

The last living weight obtained in the experiment was on one rat on the seventy-sixth day. The weight of that animal was 178 gm. This is the implied average terminal weight. The implication is false since this is only the weight of an individual animal, and the average final surviving weight of all the animals would be entirely different.

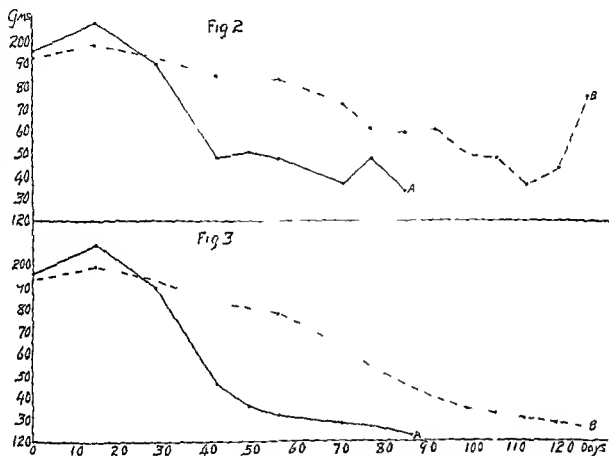
In dealing with weight data of this kind obviously there are four important points; others may appear after a more detailed study. (1) The weight of the entire unit at the start of the experiment. (2) The weight of the unit at the time of greatest gain. (3) The weight of the unit just prior to the first death. (4) The weight of the unit at the end, when all animals are dead. If the weight of the unit just before the first death (Fig. 1, point A) is significant and the dead weight of the unit (point B) is significant, then a line joining these two points must be important. A straight line joining these points (curve 2) would imply a uniform rate of weight loss for the unit, which is not true. Obviously an accurate determination of the weight between these two extremes (where all animals are living or all are dead) would include the weights of some living and some dead animals. Calculation of the average weight of the animals in the unit by this method gives a curve which reveals a rapid decline in the weight of the unit between the twenty-eighth and fifty-eighth days. This was also the period of greatest mortality in which the unit was rapidly yielding to the vitamin B insufficiency. Between the fifty-eighth and eightieth day the decline in weight was greatly decreased. The few surviving animals were near the average death weight by the fifty-eighth day, but probably were utilizing the small amount of vitamin B in the diet very economically.

A further study of the relative value of the two methods was made by applying the principles involved to a study of the growth obtained on two groups of animals, one of which (22 animals) received a vitamin B deficient diet and the other (22 animals) received a diet deficient in both vitamin B and inorganic salts. The vitamin B deficient diet was identical with the one already given in this paper. The diet deficient in both vitamin B and inorganic salts was simply a modification of the first diet in which starch was substituted for the inorganic salts.

The same general impression is obtained whether only the surviving animals (Fig. 2) are considered or the weight curve made to represent all animals whether dead or alive (Fig. 3) until the close of the experiment. The flattening of the weight curve (Fig. 2, curve A) beginning on the forty-second day of vitamin B deficiency is exaggerated when only survival animals were considered. This exaggeration is due to the death of some light animals and their withdrawal from further consideration. The sudden drop in the weight curve just

preceding the last weigh day is due to the death of one animal and its withdrawal from the group. The weight of this animal was a much greater portion of the total weight of the surviving animals than it, as an individual, was of the total number of surviving animals.

The weight curve for the group of animals receiving both the vitamin B and salt deficient (curve B) diet presents several irregularities due to death of individuals and their withdrawal from the group between the fortieth day and the close of the experiment. The large rise in the curve between the one hundred and twelfth and one hundred and twenty-sixth day is due mainly to the death of 5 animals and their withdrawal from the experiment. In this case

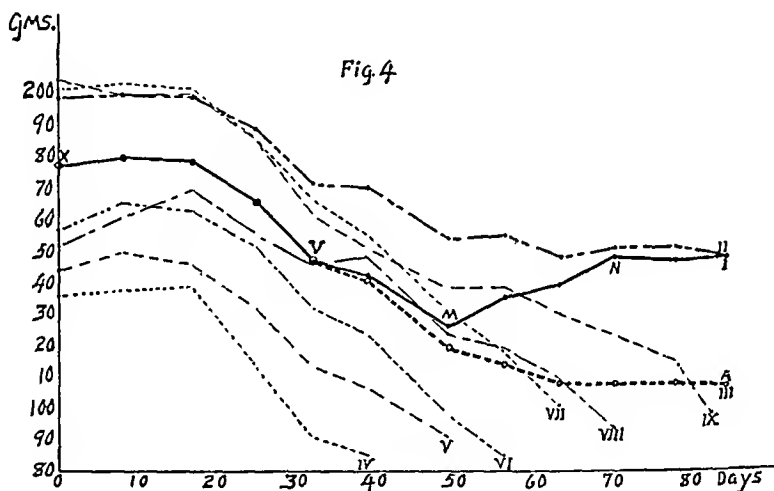


the number of animals was relatively a greater portion of the surviving animals than was the weight of these animals of the total weight of the surviving animals.

Considering the groups of animals as two units and carrying the weights of all animals whether dead or alive (Fig 3), to the close of the experiment gave smooth curves. These curves represent the condition of each experiment as a whole. It can then be said with some degree of certainty that from a study of 22 rats on a vitamin B deficient diet, the average death weight of similar animals on a diet similarly deficient in vitamin B should be about 123 gm. By the same reasoning it may be said that an individual having all the characteristics found in the 22 rats used here should approximate this weight curve if subjected to similar conditions. This is the usual meaning of the term "average curve."

A more detailed study of the methods applicable to determining weight curves was made on data obtained from animals during the first eighty five

days of experimental hyperthyroidism (Fig. 4). The diet used to produce the hyperthyroidism consisted of casein, 18 per cent; starch, 46.9 per cent; unsalted butter, 15 per cent; baker's yeast, 15 per cent; cod liver oil 2 per cent; inorganic salts (Harris), 3 per cent; and desiccated thyroid, 0.1 per cent. Thirty-four rats (17 males and 17 females) which varied in age from 177 to 288 days were used. At the start of the experiment the lowest weight (135 gm.) was that of a female. The highest weight (214 gm.) was that of a male. The average weight of all animals was 177 gm. Between the beginning and the thirty-second day of the experiment there were no deaths. During the first few days some of the animals gained and others lost in weight. The curve X to V represents the average weight of all animals before deaths began to complicate the data. Curve I represents the average weight of the animals surviving on the various weigh days. The rapid rise in this curve from M to N indicates that the



surviving animals for some reason were rapidly gaining in weight. As a matter of fact, the average weight of the 9 animals which survived the entire eighty-five days did not at any time show a significant increase in weight as revealed by curve II. The rapid rise in curve I from M to N is due to the withdrawal from the weight curve of 23 animals which died between the forty-ninth and seventieth day of the experiment. This number of animals was relatively a much larger portion of the total number of animals living on the forty-ninth day than was their weight of the total weight of the living animals.

Curves IV to IX represent subgroups of animals which were recorded dead at various times during the course of the experiment. The last weight in each curve is the average death weight of the animals represented. Curve III (X-V-A) is the weight curve of the entire group whether dead or alive. Curves III and I coincide from X to V since during this period there was only a change in weight, unaffected by deaths. From V to A, however, in curve III the experiment was considered as a unit, and the dead weight of all animals which died in the course of the experiment are carried in the curve. In this

curve there are no appreciable fluctuations which are not significant. There is a gradual decline in this curve between the seventeenth and the sixty third day, which is the general story seen in the average curves of all subgroups of animals whether they were dead on the thirty ninth day or still living at the close of the experiment on the eighty fifth day. This curve also shows that between the sixty third and the eighty fifth day there was only a slight change in the average weight of the unit which is in record with curve II representing the subgroup which lived the entire eighty five days.

SUMMARY

1 A weight curve which represents the average weight of surviving animals from time to time in the course of an experiment is criticized.

2 A method is proposed in which all animals on a given experiment are considered as a unit, and no individual animal is disregarded from the weight curve because of death in the course of the experiment.

COMING MEETINGS

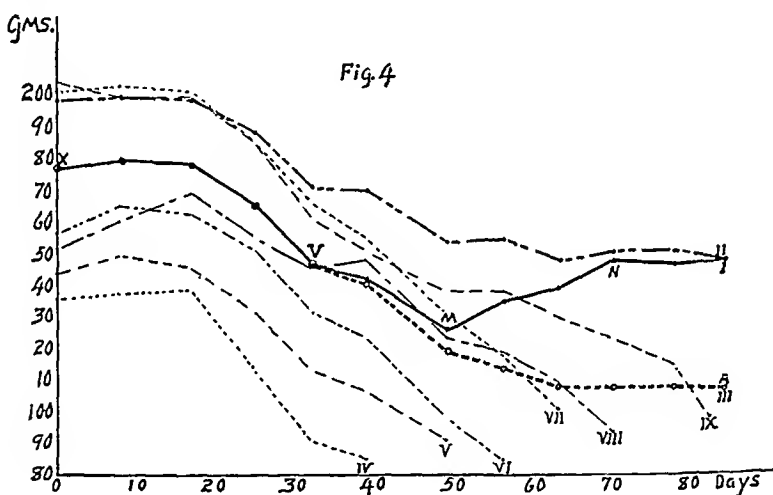
American Society of Clinical Pathologists, San Francisco June 9 10 and 11. The meeting on June 9 is devoted entirely to a tumor seminar under the direction of Dr. Lee McCarthy of Washington D. C. The subject is "Pathology of the Skin." Headquarters Palace Hotel.

The Association for the Study of Allergy, June 10 and 11. Headquarters, Hotel Empire San Francisco.

The American Rheumatism Association, June 11 at Toland Hall. The University of California Hospital San Francisco.



days of experimental hyperthyroidism (Fig. 4). The diet used to produce the hyperthyroidism consisted of casein, 18 per cent; starch, 46.9 per cent; unsalted butter, 15 per cent; baker's yeast, 15 per cent; cod liver oil 2 per cent; inorganic salts (Harris), 3 per cent; and desiccated thyroid, 0.1 per cent. Thirty-four rats (17 males and 17 females) which varied in age from 177 to 288 days were used. At the start of the experiment the lowest weight (135 gm.) was that of a female. The highest weight (214 gm.) was that of a male. The average weight of all animals was 177 gm. Between the beginning and the thirty-second day of the experiment there were no deaths. During the first few days some of the animals gained and others lost in weight. The curve X to V represents the average weight of all animals before deaths began to complicate the data. Curve I represents the average weight of the animals surviving on the various weigh days. The rapid rise in this curve from M to N indicates that the



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The patch test is not only as accurate, numerically, as the Pirquet test, but shows the following definite advantages over all tuberculin tests

1. It is painless and does not excite nervous children and a good relation between the doctor and the patient is thus retained.
2. It consumes less time.
3. It does not involve the use of instruments and their sterilization or trauma to the skin through injection, scarification or rubbing
4. It can be carried out without help, or by a nurse or an assistant.
5. In contrast to all other patch tests, it creates a sharply limited area of reaction and prevents an uncontrollable spread of tuberculin on the skin
6. It eliminates the danger of infection
7. It has never led to a local or general constitutional reaction

PNEUMONIA, Due to *B. Friedländeri*, Bullowa, J. G. M., Chess, J., and Friedman, N. B.
Arch. Int. Med. 60. 735, 1937

A report on 41 patients with acute pneumonia due to *B. Friedländeri* is presented in an attempt to portray the disease as a clinical and pathologic entity. The findings for these patients showed a marked similarity to those for patients with pneumococcal lobar pneumonia.

The mortality rate for the entire group of 41 patients was 83 per cent. The highest mortality rate (94 per cent) occurred in patients infected with *B. Friedländeri* A who were not given serum.

Serum therapy was attempted for 8 of the patients, but in only 6 of them was the serum specifically related to the infecting organism. The mortality rate for these 6 patients who were infected with *B. Friedländeri* A and who received serum was 50 per cent, as contrasted with a mortality rate of 94 per cent for the 18 patients infected with the same organism and not treated with serum.

Note. Since the preparation of this report Solomon has published reports of 5 patients from Bellevue Hospital. Only 1 patient was treated as early as the third day. The 4 others were treated on the fifth day or later. The amount of serum given was inadequate. All his patients who received serum died.

HYDATID DISEASE, Godfrey, M. F. Arch. Int. Med. 60. 783, 1937

In the course of a thorough review of this condition the author presents the following avenues of laboratory diagnosis. Three laboratory tests are of great value:

1. *The Precipitin Test*—This test, which employs the serum of the patient in prepared hydatid fluid, is accurate in 65 per cent of cases. It is of value only when used with carefully prepared controls.

2. *The Intradermal Test*—The intradermal test of Casani is very valuable. It is carried out in a manner similar to that of the Pirquet test for tuberculosis. Hydatid fluid obtained by puncture of an aseptic cyst from the lung or liver of a sheep is filtered and used for this test. When 0.3 cc. of the fluid is injected intradermally into the arm of the patient, a wheel is raised that is about 8 mm. in diameter. An injection of physiologic solution of sodium chloride should be used as a control. When the reaction is positive, the wheel reaches its maximum size of 4 or 5 cm. in from ten to twenty minutes and is surrounded by an erythematous zone. This test, according to Fairley, showed positive results in 56 per cent of the cases in which there were uncomplicated cysts and in 26 per cent of the cases in which there were ruptured or suppurating cysts. The Casani test is a useful pre-operative guide, since it indicates when there has been a dangerous degree of absorption of hydatid fluid.

3. *The Complement Fixation Test*—This test is specific and is dependent on a specific antibody in the serum of patients who have absorbed hydatid antigen. Fairley's technique is similar to that of the Wassermann test. According to Fairley and Williams, in 52.4 per

cent of cases there is a positive reaction preoperatively; whereas in 52.8 per cent of cases there is a positive result when there are residual or recurrent cysts.

Leucocyte Count.—When the common causes of eosinophilia, such as asthma, chronic disease of the skin and intestinal worms can be eliminated, a certain amount of reliance may be placed on the presence of eosinophilia. In about half the cases of hydatid disease the eosinophile count is higher than normal, the upper limit of normal being placed by Fairley and Kellaway at 300 eosinophilic leucocytes per cubic millimeter of blood (6 per cent). Rarely, the eosinophiles outnumber the neutrophils.

Characteristics of Hydatid Elements.—The hydatid fluid is clear, limpid, and alkaline, with a specific gravity of from 1.008 to 1.015. It contains about 6 per cent of sodium chloride. After centrifugation it should be examined for scolices and hooklets. A scolex measures 160 microns in length and 115 in breadth. Hooklets also may be seen, their length varying from 20 to 40 microns.

BACTERIEMIA, Significance of Hemolytic Streptococci, Keefer, C. S., Ingelfinger, F. J., and Spink, W. W. Arch. Int. Med. 60: 1085, 1937.

From the clinical study of 246 patients with bacteremia the following facts were elicited:

Bacteremia in hemolytic streptococcal infection is seen most often in patients in the first, fourth, and seventh decades. These peaks of incidence correspond to the age incidence of infections of the throat and middle ear, the puerperal infections, and cellulitis and erysipelas.

The general fatality rate was 72 per cent. It was highest for the patients with cellulitis and erysipelas regardless of age, slightly lower for those with puerperal sepsis, and lowest of all for patients less than 20 years of age with infections of the throat, middle ear, and mastoid. Aside from the differences in mortality according to age and portal of entry, such factors as the duration of the sepsis, the location of infection in an area that could or could not be treated adequately, and the presence or absence of debilitating diseases were of importance in determining the outcome.

The commonest metastatic lesions were in the joints, subcutaneous tissues, and endocardium, although only about 30 per cent of the patients showed metastases.

Recovery following bacteremia occurred after a transitory invasion of the blood from a focal lesion without metastases or when the blood stream was cleared of organisms and focal infection was established if the lesion could be treated surgically.

Death occurred among those with debilitating diseases, those with rapidly spreading infection without localization, and those in whom localization occurred in an area which could not be treated surgically (peritoneum, endocardium, or meninges). Sometimes the blood was cleared of organisms, but the foci of infection were situated so that they could not be treated adequately.

From a study of the organisms which cause bacteremia and the immune reactions of patients with and without bacteremia, the following statements are justified:

Organisms isolated from local lesions and from the circulating blood were beta-hemolytic streptococci. They all belonged to group A of Lancefield, and they all produced fibrinolysin.

Organisms isolated from the circulating blood frequently resisted phagocytosis and were not killed by blood from normal persons. There are exceptions to this observation, but, by and large, it is true.

Organisms isolated from local lesions were phagocytosed by the leucocytes of some normal persons and killed in varying numbers by those of different persons. The blood of patients with local lesions usually possesses the capacity of killing the homologous organisms, and this function increases with the course of the disease. The presence of circulating antibodies assists in localizing the infection and in preventing bacteremia.

In patients with bacteremia antibodies develop which aid in the clearing of the blood stream. In the author's experience all the patients with bacteremia who recovered had demonstrable antibodies in the circulating blood, and it was shown that antibodies were present in some of the patients who died, although the titer was low.

Bacteraemia in hemolytic streptococcal infection is of greater value in prognosis than in diagnosis, and its presence may be taken as indication of a loss of equilibrium between the local defense mechanism and the normal clearing mechanism. The presence of specific antibodies plays an important part in preventing bacteraemia and in clearing the blood of organisms once it has become invaded by organisms from the local focus.

HODGKIN'S DISEASE, The Etiologic Relation of the Eosinophile to the Gordon Phenomenon, Turner, J C, Jackson, H, Jr Parker F Jr Am J M Sc 195 1 1938

In a series of 11 cases of Hodgkin's disease the Gordon test has been positive only when eosinophiles could be found in the corresponding microscopic sections. The number of these cells was found to parallel the rapidity of development of the pathogenic substance upon which the positive test depends.

The pathogenic agent described by Friedmann and Elkeles in myeloid tissues has been shown to be present only in those suspensions of leucocytic cream that contain more than a certain number of eosinophiles.

Gordon's agent and Friedemann's agent would both, therefore, appear to be derived from the eosinophile and consequently are probably identical.

The Gordon test is positive in Hodgkin's disease only by virtue of the presence of eosinophiles in the lesions of this disorder.

PNEUMONIA, LOBAR, in Children Andrews E T Am J Di Child 54 1280 1937

Comparison of numerical with causative frequencies of types of pneumococcus which have been recovered from children with lobar pneumonia led to their separation into three etiologic groups, the distribution of which among patients with and without pneumonia and among other members of the same families indicated a corresponding diversity in their epidemiologic significance.

A types appeared causative in all patients with lobar pneumonia from whom they were recovered, and they were associated with a relatively high incidence of pneumonia in infected persons, high mortality among infants, marked evidence of familial dissemination when the concurrent pneumonia was ascribed to these types and relatively low incidence in families without pneumonia or in those in which the patients did not show the same types of pneumococcus.

Intermediate or B types appeared causative in many, but not all of the children with lobar pneumonia from whom they were recovered but, in comparison with the former group, showed a lower incidence of this disease in infected persons, lower mortality among infants, less evidence of familial dissemination when the concurrent pneumonia was ascribed to these types, and a relatively high incidence in families without pneumonia or in those in which the patients had pneumonia ascribed to heterologous types.

The least active types (group C) appeared to be responsible for lobar pneumonia in few of the children from whom they were recovered. Evidence for their familial dissemination was uncommon but these were the types most often recovered from persons coming in contact with patients whose pneumonia was ascribed to heterologous types.

The incidence of pneumococci, the occurrence of lobar pneumonia in infected persons, and the familial dissemination of causative types also varied with age.

Children exposed to pneumonia ascribed to A types yielded pneumococci found in the patients relatively more often than did adults.

Patients with pneumonia ascribed to A types constituted the most active sources of infection by contact.

Repeated cultures of material from the throat were helpful in revealing the probably causative types in children with lobar pneumonia.

YAWS, Comparative Histology of Syphilis and, in Jamaica, Ferris H W and Turner, T B Arch Path 24 703, 1937

The initial lesion and the fibrosiomas of yaws are characterized by epithelial hyperplasia with exudation of polymorphonuclear leucocytes. There are many lymphocytes and

plasma cells in the dermis. The scaly macules and folliculopapules have little proliferation of epithelium and only scant infiltrations of lymphocytes and plasma cells in the dermis. In the late lesions of yaws there is usually ulceration, with varying degrees of epithelial hyperplasia.

With silver preparation, the treponemes of yaws (*T. pertenue*) are constantly found in large numbers in the epidermis and more superficial dermis of the frambesiform lesions and may be numerous in the initial lesion. They are demonstrated in small numbers in the hair follicles and dermis of about 50 per cent of the folliculopapules and scaly macules of yaws. They are occasionally recognized in small numbers in late lesions.

Spirochetes are demonstrable in about 70 per cent of the papules and maculopapules of syphilis.

There is no evidence that in either yaws or syphilis the spirochetes are phagocytosed by polymorphonuclear leucocytes or histiocytes.

There is no correlation between the titer of the Wassermann reaction of the blood and the presence or number of spirochetes in the cutaneous lesions of yaws.

Histologic criteria for the differentiation of the cutaneous and subcutaneous lesions of yaws and syphilis are in general unreliable.

SHELL-FISH POISONING, Paralytic. Sommer, H., and Meyer, K. F. Arch. Path. 24: 560, 1937.

The authors have tabulated and discussed 243 cases of paralytic shellfish poisoning, with sixteen deaths, that occurred between Ventura County, Calif., and Juneau, Alaska, from 1927 to 1936. Of these, 234 cases were caused by the coast mussel; 9 were caused by the Washington clam.

Methods for detecting and quantitatively estimating the poison in shellfish have been worked out. Mussels from numerous places between central California and southern Oregon have been analyzed and toxin curves constructed for the past nine years. The curves for mussels, sand crabs, and Washington clams from localities near San Francisco since 1931 have been specially detailed. Besides the shellfish mentioned, seven of the common varieties of edible clams may contain the poison in smaller amounts.

Poisonous mussels may in no way be distinguished from normal ones, except by the animal test. Mussels subjected to various conditions in the laboratory have never shown an increase in toxicity; they usually show detoxification, the rate of which has been determined. Mussels may take up poisons from sea water.

Strong evidence has been presented which points to the water of the open ocean as the carrier of the poison. Owing to the strong adsorption of the substance on base-exchanging silicates of the sand, it is not likely to occur free in the water. The poison has been demonstrated, at least during the poison season, in the residue from filtration of sea water. Whether it is contained in the plankton or adsorbed in the microscopic sand cannot at present be decided.

Four different principles toxic for mice have been demonstrated in acid alcoholic extracts of shellfish and plankton. The methods follow:

Standard Test.—The shellfish are opened by cutting the posterior abductor muscle, the digestive glands are carefully removed and without the addition of shell water or of other tissues, are ground up thoroughly with acid methyl alcohol (4 c.c. of concentrated hydrochloric acid per liter). Several portions of the liquid are used, so that the residue becomes nearly colorless. The combined residue and liquids, which measure approximately from 60 to 80 c.c., are poured into a centrifuge tube. The mixture is brought to a boil by immersion of the tube in hot water and is kept boiling for a few minutes. Next it is gradually cooled to room temperature, preferably in the same water-bath. (If necessary the extract may be cooled rapidly and worked up at once, or it may be stored for several days. It should, however, not be kept longer than a week.) After standing for a few hours or overnight, most of the alcohol-insoluble substances have settled; they are removed by centrifugation, and the clear dark-colored fluid, or an aliquot part, is poured into a weighed glass dish and

evaporated on a boiling water bath. The amount of fluid chosen depends on the time taken for evaporation, i.e., not more than from thirty to forty five minutes, and the amount of residue expected, 200 mg is sufficient for a test, from 50 to 200 mg is usually obtained from a mussel. The residue is removed from the water bath before it is entirely dry, i.e., when it is still of a pasty consistency. It is triturated with the help of a glass rod, with several small portions of chloroform, until the latter comes off nearly colorless. The solvent is poured off each time and discarded. This extraction of the lipids and pigments may be readily recompensated if the residue shows the degree of dryness mentioned. Floating particles may, however, be made to settle by the addition of ether. The residue is next heated on the water bath for one or two minutes, with constant stirring, until it is quite viscous. It is hygroscopic and should be weighed as soon as cold. For the injections distilled water ten times the weight of the residue is added, making a solution of approximately 10 per cent.

It is evident that the method can be modified and adapted to specific needs. For the detection of mere traces of poison it may be considered advantageous to evaporate in vacuo, better yet, the poison may be precipitated from the alcoholic solution by the addition of from 5 to 10 volumes of ether. The dried residue may then be directly dissolved for injection. On the other hand, certain errors must be avoided. Grinding of the tissue with sand as performed in 1927, is of decided disadvantage since it has been shown that the shellfish poison is adsorbed by sand in much the same way as by permutit (in artificial sodium aluminum silicate). If large amounts of extract have to be injected so that it becomes desirable to neutralize the solution, great care must be exercised in doing this, since even a slightly alkaline reaction may destroy 50 per cent of the poison in a short time. In the standard test as done at present the solution is not neutralized for injection. The thorough extraction with chloroform and the subsequent heating should be ample to remove the free hydrochloric acid introduced. It is very likely that the remaining acid is bound to betaine which is found in large amounts in any extract of shellfish and which is quite harmless on intraperitoneal injection in the form of its hydrochloride. A fatality from the acid reaction alone has never been encountered.

The average lethal dose of shellfish poison has been defined as that amount which will kill a 20 gm mouse in from ten to twenty minutes, with typical neurotoxic symptoms. Besides unresponsiveness, a wobbling gait, strong spasms and gaspings, the heart block may be used as a differential symptom. It is present only when the dose of poison is not too massive, otherwise, the heart is entirely stopped. It develops soon after the heart has been exposed and may last for as long as half an hour. The intestinal peristalsis also is invariably pronounced on autopsy immediately after death from pure mussel poison.

Field Test—Another test for shellfish poison, which is of great help in routine examinations, consists in extraction of the livers with boiling acidulated water, cooling, and injection of 1 cc of the supernatant liquid. It has been used as a field method when rapid examination of several mussel beds became imperative for the establishment of quarantine measures. For an average sized mussel liver, 1 cc of tenth normal hydrochloric acid plus 9 cc of water was used. Thorough grinding of the tissue is essential in this test also. One minute of boiling is usually sufficient to coagulate the bulk of the insoluble material, the acid need not be neutralized before injection. Owing probably to the inclusion of poison in the precipitated protein, this test shows about half the number of average lethal doses per mussel expected according to the standard test. The ratio is still more unfavorable when neutral extracts are compared.

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While the author states in his preface that the purpose of the book is "to present the subject of hematology in a manner acceptable to practising physicians as well as to those primarily interested in the study of disease by laboratory methods," his book will appeal to the physician rather than to the clinical pathologist or hematologist already familiar with the subject.

The chapter on laboratory technique is too brief to add materially to the value of this text, and while their application to the study of anemias is obvious, tests for free hydrochloric acid in gastric contents, urobilin in the urine and the occult blood in the feces appear somewhat out of place in a book concerned with hematology. The general character of the book is such as to indicate that it was prepared primarily with the clinician in view, and to those who are desirous of becoming informed upon the present views of disorders of the hematopoietic system it should prove of interest and value. It cannot, however, be regarded as an exhaustive discussion of the subject.

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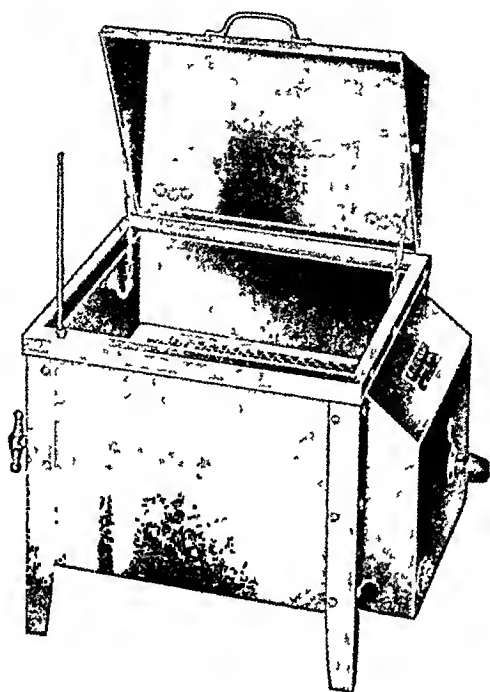
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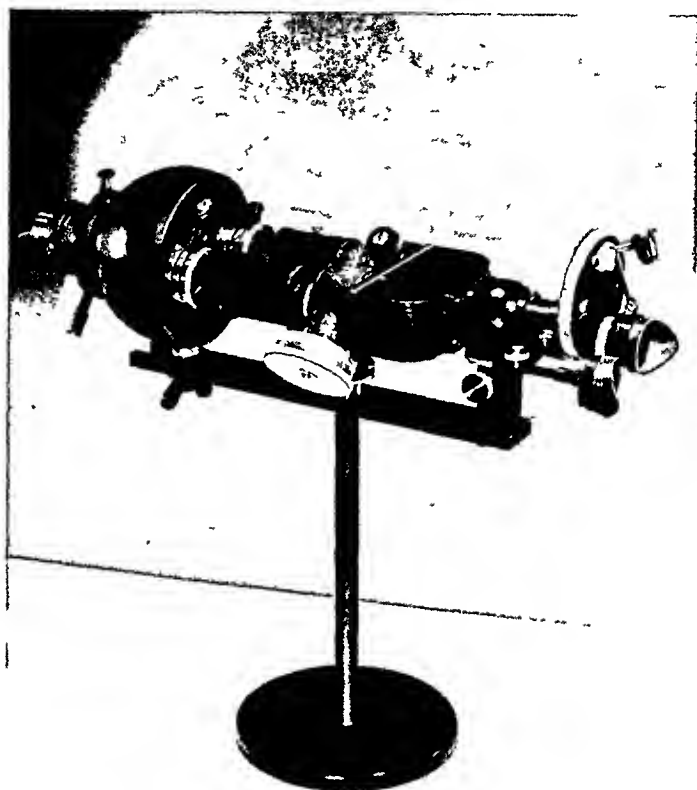
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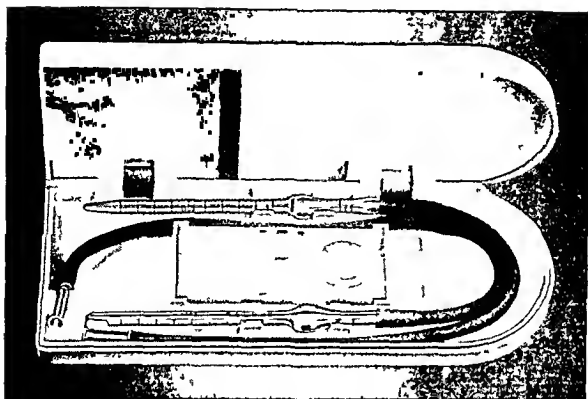


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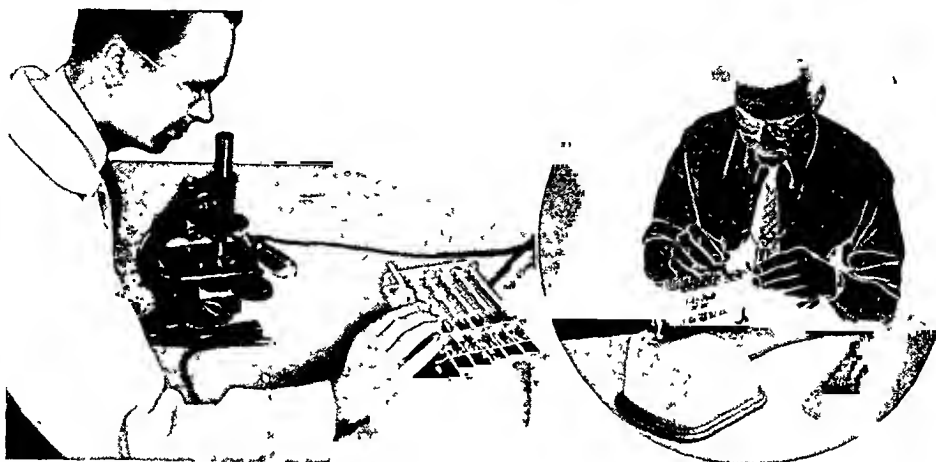
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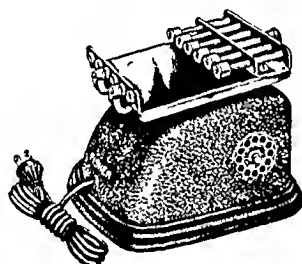
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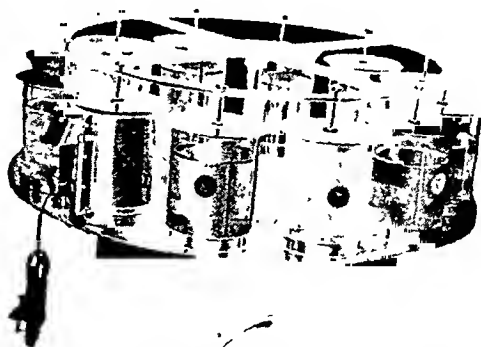
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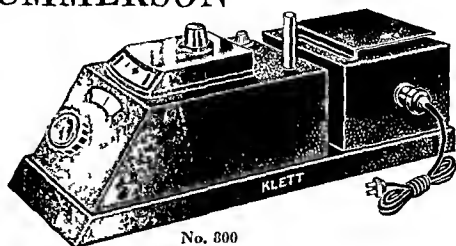
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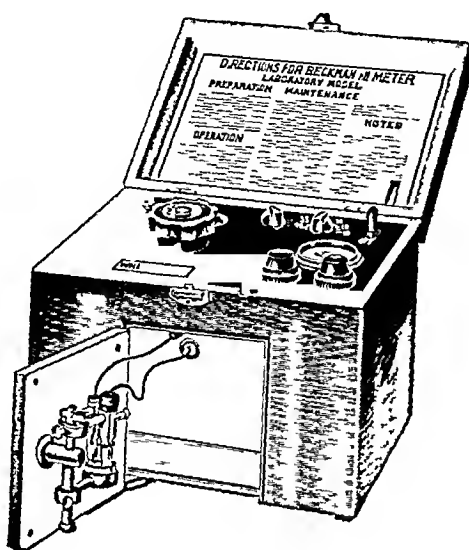
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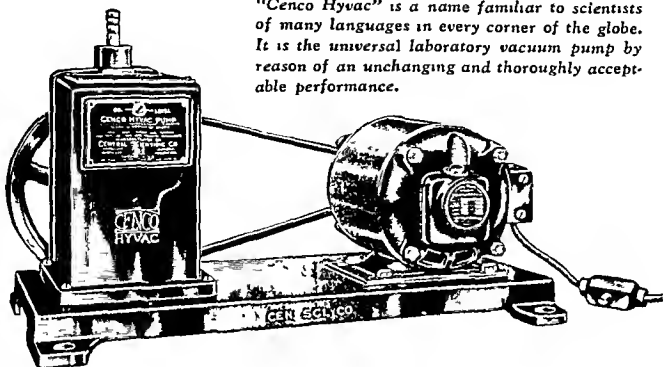
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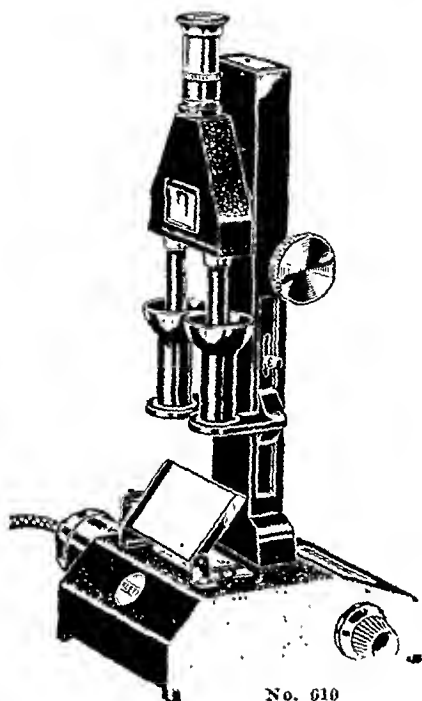
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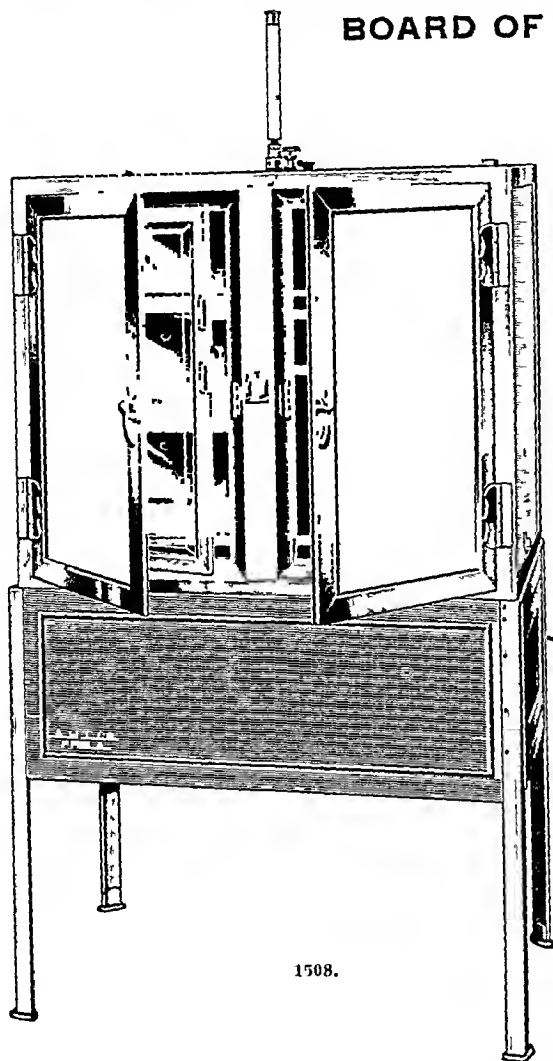
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The Journal of Laboratory and Clinical Medicine

VOL 23

JUNE 1938

NO 9

CLINICAL AND EXPERIMENTAL

THE TAKATA-ARA REACTION*

I. CLINICAL SIGNIFICANCE

JULIUS CHASNOFF, M.D., AND SYDNEY SOLOMON, M.D., NEW YORK, N. Y.

DURING the past year, the Takata-Ara test was performed on a series of 75 cases. Our purpose was to determine what diagnostic value the test has in cases of liver disease. With that in view, the reaction was determined in a group of patients with outspoken liver disease, and in a group in which there was no clinical reason to suspect any liver involvement. The latter were intended to serve as controls.

HISTORY

In 1925 Takata¹ devised a test depending on a colloidal reaction which he felt was of value in differentiating between lobar and bronchopneumonia. In the same year Takata and Ara² using cerebrospinal fluid, reported further experiences with the test, which suggested its use in differentiating between meningitis and syphilitic involvement of the central nervous system. These investigators attributed the flocculation which occurs in the positive test to a change in the relationship of the albumin and globulin in the serum or spinal fluid.

*From the service of Dr. I. W. Hill, Beth Israel Hospital, New York.
Aided by a grant made by the late Henry Dazian.

Received for publication Oct. 29, 1937.

In 1929 Nicole,³ under the direction of Staub, showed that a positive Takata-Ara reaction in spinal fluid occurs when the albumin-globulin ratio is changed, particularly the globulin. In 1924 Adler and Strauss⁴ had shown that in cases where there was liver damage, the relationship of the serum albumin and globulin was disturbed. Having these facts in mind, Stanb⁵ suggested that the Takata-Ara reaction might be of value as a diagnostic measure in liver disease, and actually found the test positive in all cases with severe parenchymatous liver damage. Influenced by Staub's suggestion, Jezler⁶⁻⁸ performed the test on serum and ascitic fluid, and reported positive results in cases of severe parenchymatous disease of the liver, particularly cirrhosis.

Since then numerous conflicting reports have appeared endeavoring to assay the value of the test as a diagnostic measure in liver disease. Greatest attention has been given to the results of the reaction in cases of cirrhosis. Most investigators, among them Stanb,^{5, 9} Skouge,¹⁰ Oliva and Pescarmona,¹¹ Schindel and Barth,¹² and Heath,¹³ have obtained positive results in a large percentage of their cirrhosis cases. Takata,¹⁴ in a monograph written in 1935, found that of all the cases reported in the literature the test was positive in 90.1 per cent and negative in 9.9 per cent of cases of liver cirrhosis. Similarly, Kirk¹⁵ in 1936 collected from the literature 375 cases of liver cirrhosis of which 315 gave a positive Takata-Ara reaction.

On the basis of reports similar to these, some have concluded that the test is fairly specific for cirrhosis of the liver. However, most of these authors agree that in the early stages of the disease the test is frequently negative. Crane¹⁶ found the test to be highly specific in cirrhosis, but also in cases of acute yellow atrophy; and many authors^{9-11, 17} have reported positive results in various other diseases associated with liver damage. On the other hand, Neuweiler¹⁸ found that in toxemia of pregnancy, even when there was severest liver change, the test was always negative. Fulde¹⁹ had similar results. Ragins,²⁰ however, did obtain positive results in cases of toxemia of pregnancy.

Oefelein,²¹ Ragins, and others report positive reactions in cases of liver disease in which the degree of liver damage is not always extensive, such as malignant involvement of the liver, stasis due to cardiac failure, hepatocellular (catarrhal) jaundice, and liver damage secondary to cholelithiasis with obstruction.

Different results have been obtained in carcinomatous involvement of the liver. Schindel and Barth, van Ginkel,²² Magath,²³ and Kirk have found that the test may be positive in liver malignancy. On the other hand, Jezler, Crane, and Hugonot and Solier²⁴ obtained negative results in this condition. Fulde claims that the test is *always* negative in carcinoma, even with extensive involvement of the liver where very little functioning liver tissue remains. He concludes, therefore, that the test is of absolute value in differentiating between carcinoma involving the liver and cirrhosis. Since this differential problem not infrequently confronts the clinician, such claims for the test become of great importance and demand very critical consideration.

METHOD AND INTERPRETATION OF REACTION

We employed Crane's modification of the method used by Jezler. In every case the test was performed on the serum within one to two hours from the time the blood was drawn, although according to Jezler,⁷ Crane, and Kirk serum kept in the refrigerator or preserved with toluol or thymol can be used after several days or even weeks.

One cubic centimeter of 0.9 per cent solution of sodium chloride is placed into each of six small tubes. To the first tube 1 cc of serum is added. After mixing with the saline 1 cc of the mixture is removed and added to the saline in the second tube. This procedure is continued throughout the six tubes, 1 cc of the mixture from the last tube being discarded. The six tubes now contain serum dilutions of 1:2 to 1:64. To each of the tubes there is then added 0.25 cc of 10 per cent sodium carbonate. After shaking, 0.15 cc of 0.5 per cent mercuric chloride is added. The tubes are thoroughly shaken and examined after five minutes and again after twenty-four hours. A positive reaction is indicated by a pearly flocculum. Occasionally a reddish precipitate occurs which is not a true flocculum and should not be considered as representing the specific reaction.

Our manner of interpreting the results was as follows:

1 Strong positive—A definite flocculation in 4 or more tubes or in 3 tubes at least one of which shows a flocculum occupying about one third of the column.

2 Positive—A definite but small flocculation in 3 or 4 tubes.

3 Weak positive—A definite but minimal flocculation in no more than 3 tubes.

All others were considered negative.

There is a difference of opinion among various authors as to the extent to which the readings of the reaction may be subdivided. Heath divides the readings into five groups. Magath also lists five groups, but actually considers those falling into the group he terms "suspicious" as negative. He states that "from a technical standpoint it is impossible to identify the test accurately as positive unless a degree of precipitation is present sufficiently great to leave no doubt about the positiveness of the reaction." In our series, we regarded as negative a minimal flocculation in one or two tubes which Magath considers weakly positive.

RESULTS

The test was performed 82 times on the 75 cases. Table I is a summary of the results obtained in the entire series. Of special interest to us were the results obtained in liver cirrhosis, malignancy involving the liver, and liver enlargement due to cardiac failure. Of the 7 cases of clinical cirrhosis, 2 were regarded as of syphilitic origin. In the other 5 cases the diagnosis of true Laennec cirrhosis was made. In one of the cases of Laennec cirrhosis our first

TABLE I
RESULTS OF THE TAKATA-ARA REACTION IN 75 CASES

DISEASE	NO. OF CASES	NO. OF TESTS	POSITIVE REACTIONS			TOTAL POSITIVES	NEGATIVE REACTIONS
			STRONG	POSITIVE	WEAK		
Cirrhosis:							
Laënnec	5	8	4	3	0	7	1
Syphilitic	2	4	1	2	0	3	1
Carcinoma of the liver	7	8	3	1	4	8	0
Parenchymatous liver damage:							
Hepatocellular jaundice	4	4	0	0	1	1	3
Syphilitic hepatitis	1	1	0	0	1	1	0
Acute toxic hepatitis	1	1	0	0	1	1	0
Liver abscess	1	1	0	0	0	0	1
Obstructive jaundice:							
Stricture of common duct	1	1	0	1	0	1	0
Calculus in common duct	3	3	0	0	0	0	3
Adenocarcinoma of common duct	1	1	0	1	0	1	0
Adenocarcinoma of pancreas	3	3	0	0	1	1	2
Hepatomegaly of unde- termined origin	8	8	0	2	1	3	5
Cardiac decompensation with permanent liver enlargement:							
Arteriosclerotic and/or hypertensive heart disease	8	8	0	0	2	2	6
Rheumatic	2	2	0	0	1	1	1
Thyrototoxic	1	1	0	0	0	0	1
Miscellaneous	27	28	0	6	1	7	21
Totals	75	82	8	16	13	37	45

result was negative. Exactly one month later the reading was strongly positive. In one case of syphilitic cirrhosis the test was at first negative, but 4 days later it was strongly positive.

In 6 of the cases with ascites, the Takata-Ara reaction was performed on the ascitic fluid. Two of these gave a positive reaction in both the blood and ascitic fluid. In two other cases, although the ascitic fluid gave a positive reaction, the degree of flocculation was not as marked as in the blood. In one instance, the reaction in the blood was strongly positive, the ascitic fluid negative. In the sixth of this group, the blood was at first negative, and at that time the ascitic fluid was also negative. On a second examination, when the reaction was strongly positive in the blood, the ascitic fluid was still negative. On a third determination the reaction in the blood was still positive and that in the ascitic fluid had become weakly positive (see Table II).

From Table I it will be noted that all of the 7 cases of liver malignancy gave positive reactions. Of the 8 tests done, 3 were strongly positive, 1 positive, and 4 weakly positive. In this group the diagnosis was confirmed in 3

TABLE II

RELATIONSHIP BETWEEN TAKATA REACTION IN BLOOD AND ASCITIC FLUID

CASE NO	TAKATA IN BLOOD	TAKATA IN ASCITIC FLUID
3	+++	++
7	+++	0
10	0	0
	+++	0
	+++	+
15	++	++
68	++	++
70	++	+

+++strong positive

++positive

+=weak positive

0=negative

by autopsy or operation. In the other 4, the fact that the site of a primary lesion was known and the hard nodular state of the liver left little doubt as to the diagnosis of carcinoma of the liver.

Under the head of parenchymatous liver disease, it will be noted that of the 4 cases with hepatocellular (catarrhal) jaundice, one gave a positive reaction. Each of our 2 hepatitis cases, one syphilitic and the other acute toxic, gave positive reactions.

We had in our series 11 cases of long standing liver enlargement in cardiac patients who were chronically decompensated. These may justifiably be assumed to be cases of cardiac cirrhosis. Of this group, 3 gave positive reactions, 8 negative.

It is of interest to note that of our 27 miscellaneous cases, serving as controls, 6 gave a positive reaction. The diagnoses in these cases were senile catarract, chronic myelogenous leucemia and bronchopneumonia, chronic glomerular nephritis, peritoneal carcinomatosis, lobar pneumonia and asthma, chronic adnexal disease. In none of these was there any clinical evidence of liver cell damage, although in the first three mentioned, the liver was enlarged. However, it should be noted that there were 3 other cases with liver enlargement in this miscellaneous group in which the Takata reaction was negative.

COMMENTS

Our findings are in accord with those of most investigators who claim that the test is positive in advanced cirrhosis, but may be negative in early cirrhosis. However, we must point out that in only two of our cases of cirrhosis did we obtain a negative reaction first and a positive reaction later. But, since the time interval between the two tests was in the one instance a period of one month and in the other only four days, it is unwise to attempt to attribute this change to a progression of the liver disease from an early to an advanced stage. Furthermore, in both instances, when the test was negative, the patient clinically showed evidences of advanced hepatic cirrhosis. We had a further experience of obtaining a weak positive reaction in a patient with a peptic ulcer, in whom the reaction was negative one week later. This difference in the results obtained would rather indicate the unreliability of one determination in any suspected case. Fulde reports a case in which this is demonstrated even more clearly. The case, according to clinical criteria, was definitely one

of liver cirrhosis. The test performed on two successive days was negative the first day and weakly positive the next. As a diagnostic test in cirrhosis, therefore, the Takata-Ara reaction is of little value, since by the time the reaction becomes positive the diagnosis can usually be made clinically.

Even with this fact in mind, the test would still be of considerable value if the contention of Fulde and others could be substantiated that it is negative in metastatic liver involvement and therefore can be used to differentiate between cirrhosis and carcinoma, where this differentiation presents difficulties clinically. In our series, however, the test was positive in all cases in which there was any degree of certainty about the diagnosis of carcinoma. We can, therefore, not subscribe to the view that the test is of differential diagnostic value. The results of others would also indicate that the test may be positive in malignancy. In Kirk's series, for example, of 5 cases with liver metastases, 3 gave a positive reaction. Of the 133 cases of tumor of the liver which he gathered from the literature, over 30 per cent gave a positive Takata-Ara reaction. Magath similarly reports 7 positive reactions out of 9 cases of carcinoma involving the liver. Schindel and Barth obtained 8 positive reactions in 9 cases of liver malignancy.

Along with Fulde, we have found that a small percentage of cases with liver enlargement due to cardiac failure show a positive Takata-Ara reaction. This has been the experience of many investigators. In the 3,583 cases collected from the literature by Kirk, 122 were cases of passive congestion of the liver and 20 per cent of these showed a positive Takata-Ara reaction.

The results in other parenchymatous affections of the liver are inconstant. The test is positive in some cases of hepatocellular jaundice and negative in others. In obstructive jaundice we found the test negative 5 times and positive in 3 cases. Magath reports a negative reaction, except where extensive liver damage was present. We cannot agree with Oefelein that the test is such an extremely sensitive indicator of the condition of the liver parenchyma that it will register even small changes. By plotting the amount of flocculation observed in each tube, he obtains various types of curves and feels that the type of curve is indicative of either progress or regression of the disease process in the liver. Magath finds that the degree of positiveness is not well correlated with the degree of liver disease and states that "it is evident that the subdivisions of the test are too fine for clinical use."

The fact that positive reactions were obtained in 6 cases where there was clinically no liver involvement is significant in that it supports the view that the test is by no means a specific one. It was found by others to be positive in a variety of nonhepatic diseases, for example, syphilis,^{22, 25} pulmonary tuberculosis,²⁶ glomerular nephritis,²⁷ infectious diseases,^{20, 24} and protozoal infestations of the blood.²⁴ Four out of 33 patients with no evidence of liver disease in Magath's series gave a positive reaction, and Kirk obtained similar results in 7 out of 39 patients. Heath showed that 3 per cent of general medical cases in which liver damage was not diagnosed gave positive reactions. Findings such as these have led Jezler, Magath, Kirk, and many others to the conclusion that the test is not specific for cirrhosis of the liver.

Some investigators who have performed the test on the ascitic fluid as well as on the blood serum, feel that the reaction in both media runs parallel. Staub and Jezler²⁸ go further and claim that the test can be performed with equally reliable results on various body fluids for example the contents of blisters, pleural exudate, and knee joint effusions (although they did not obtain positive results in the spinal fluid of cirrhotics even when the blood was positive). In those cases, however, in which we performed the test simultaneously on the ascitic fluid and blood serum, we frequently found a discrepancy between the reactions obtained in the two media.

CONCLUSIONS

- 1 The Takata-Ara reaction is positive in cases of advanced liver cirrhosis.
- 2 It is also positive in most cases of malignancy involving the liver.
- 3 The test is, therefore, not a satisfactory means of differentiating between cirrhosis of the liver and carcinoma involving the liver.
- 4 The test is frequently positive in severe liver damage and may be positive in mild parenchymatous disease of the liver and in cases where the liver is enlarged due to cardiac failure.
- 5 The test is occasionally positive in cases in which there is clinically no evidence of liver disease.
- 6 The Takata-Ara reaction cannot be regarded as a specific test for cirrhosis of the liver, but a negative reaction in any suspected case would tend to cast doubt on the diagnosis.

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THE TAKATA-ARA REACTION*

II. MECHANISM—WITH SPECIAL REFERENCE TO THE INFLUENCE OF THE AMMONIA BLOOD LEVEL

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SINCE the introduction of the Takata-Ara test there has been considerable speculation as to the mechanism of the reaction. The test depends on an alteration in the stability of a colloidal suspension of a mercury oxysol, resulting in the formation of a pearly flocculum.

Various explanations for this flocculation have been offered. The one most generally accepted is that held by the originators of the test, Takata and Ara,^{1,2} according to whom the formation of the flocculum depends upon an alteration in the protein constituents of the blood serum. Some investigators believe that the factor of primary importance is the lowered albumin, while others take the view that the elevated globulin is the responsible agent. More recently the reaction has been attributed to various other factors, for example, the total proteins, fibrinogen, certain fatty acids, heparin, the hydrogen ion concentration, and the ammonia level in the blood.

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In 1935 Staub and Jezler,³ in order to prove that the result of the Takata Ara reaction is dependent upon the colloid make up of the serum, performed an interesting series of experiments. They passed Takata positive sera through Bechhold filters and found that the filtrate gave a negative reaction, while the residue continued to be positive. They concluded from this that the flocculation reaction was dependent upon the colloidal residue. Further, they warmed Takata positive sera till clot formation occurred and then filtered and obtained a negative reaction in the filtrate. They also found that a pure 2 per cent globulin solution, prepared from ox blood always gave a positive reaction.

The evidence for the claim that an altered albumin globulin ratio is responsible for the Takata Ara reaction comes from various sources. Takata, Nicole,⁴ and Jezler³ showed that a positive reaction was obtained in cases in which this shift in the albumin globulin ratio occurred. Jezler found an increased globulin in all of his Takata positive sera. Jezler and Merez⁵ further demonstrated that in almost all positive cases there was an elevation of the tryptophan content of the serum above the upper limit of normal. Since globulin contains three times as much tryptophan as does albumin, they concluded that this was corroborative evidence of an elevation of the globulin fraction. This is stressed by most investigators who subscribe to the theory that the altered albumin globulin ratio plays an important rôle. On the other hand, Tannenholz⁷ feels that the albumin serves as a protective colloid to hinder the formation of a mercury precipitate in normal serum. Oliva and Pescarmona⁸ from their work also concluded that the reaction depended on a diminution in the protective action of serum albumin. Abrams and Robert Wallach⁹ and most of the Italian school take the same view.

Adler,¹⁰ in a monograph on plasma and serum, quoted the normal albumin globulin figures obtained by various investigators in a large variety of animals. These showed a wide range of normal values. Staub and Jezler performed the Takata Ara reaction on a small group of these animals and, using Adler's figures, demonstrated a direct relationship between the degree of positivity of the Takata Ara reaction and the relative globulin content of the blood in all but the frog. Their results are so striking that their table is reproduced here (Table I).

TABLE I
ALBUMIN GLOBULIN RATIO AND TAKATA REACTION IN VARIOUS ANIMALS

ANIMAL	TAKATA ARA REACTION	ALBUMIN GLOBULIN RATIO
Guinea Pig	0	2 4/1
Pig	0	1 5/1
Man	0	1 5/1
Sheep	(+)	?
Cow	+	0 8/1
Horse	++	0 6/1
Frog	0	0 2/1

Most of the cases reviewed by Staub and Jezler did show the albumin globulin ratio below normal but only one third showed a ratio of less than one. The recent investigations of Magath¹¹ and Kirk¹² support the view that the shift in the albumin globulin ratio is in large measure responsible for the positive

reaction obtained. Magath found such a shift in 12 out of 21 cases with a positive Takata-Ara reaction. Kirk obtained positive Takata-Ara reactions in 13 out of 18 cases showing altered ratio. The latter goes further and agrees with Jezler⁵ and Lazzaro¹³ that there is a definite relationship between the positive Takata-Ara and an increase in the globulin fraction. He found that in only 3 of his 22 cases with a positive reaction was the globulin fraction less than 3, and that of 20 sera in which the globulin was 3 or over only one gave a negative Takata-Ara reaction.

Some investigators have presented evidence which casts some doubt on the existence of a parallelism between the Takata-Ara reaction and the altered albumin-globulin ratio. In 2 of Magath's cases, despite the reversal of the albumin-globulin ratio, the reaction was negative. Lazzaro also occasionally obtained a negative Takata-Ara reaction with a low ratio. Skouge¹⁴ found this to be true even more often. Schindel and Barth,¹⁵ in only 7 of 21 positive sera, obtained albumin-globulin ratios of less than 1. Fulde¹⁶ feels that there is no parallel between the result of the reaction and the degree of increase in globulin and suggests that the shift in the albumin-globulin is only one of the factors responsible for a positive Takata-Ara reaction.

Abrami and Robert-Wallieh frequently found a decrease in the value of the total protein in Takata-positive sera. However, Schindel and Barth failed to find a relationship between the Takata-Ara reaction and the total serum protein. Hugonot and Sohier¹⁷ maintain that a positive Takata alone is not as indicative of liver damage as when associated with a diminution in the total serum protein. Staub and Jezler deny this contention, and Fiessinger and Gothie¹⁸ failed to observe either constant or significant alterations in the serum protein in dogs following Eek fistulas or hepatectomy.

In an effort to determine the influence of proteinemia on the Takata-Ara reaction, Staub and Jezler performed several experimental studies. They endeavored to bring about a positive reaction in people with healthy livers by means of a diet rich in protein. Five subjects were fed a breakfast containing 70 to 100 grams of protein. Four hours later the blood was examined with negative results. They also attempted to reverse positive Takata reactions by feeding abundant carbohydrates. However, it appears questionable whether a diet containing such quantities of protein would be adequate to effect any appreciable change in the total protein of the serum of normal individuals. Magath states that there is no evidence to indicate that changes in the total protein can be correlated with the Takata-Ara reaction. He suggests that the fibrinogen content of the serum may influence the reaction, thus accounting for Takata's positive results in cases of pneumonia.

The suggestion that low fatty acids are responsible for the Takata-Ara reaction has been under investigation. This work was initiated by the findings of Kállós-Deffner.¹⁹ In 1933, working with rabbits, she demonstrated that during starvation, or when the animals were kept in a rarefied atmosphere for a long time, large quantities of acetone and B-hydroxybutyric acid appeared in the blood. Her further studies showed that while this ketonemia was present the Takata-

Ara reaction was positive and that with its disappearance the reaction became negative. Sehndel²⁰ found that the addition of low fatty acids to Takata negative sera rendered the reactions positive and expressed the belief that positive reactions were dependent upon the presence in the blood of such fatty acids, occurring as intermediate products of metabolism in liver disease. Ueko,²¹ and Takata²² himself do not subscribe to this view. The former feels that the flocculation following the addition of low fatty acids is merely due to acidification and disturbance of the mercury salts. Staub and Jezler also reject Sehndel's contention. They determined the ketone bodies in the blood of many diabetics with marked ketonuria and compared their findings with the results of the Takata Ara reaction. They could not find the slightest relationship between the two. The fact that cases in diabetic coma never gave a positive reaction was to them further strong evidence against the fatty acid theory.

Zirm,²³ Medvei and Paschikis²⁴ and Ragins²⁵ have found that the addition of heparin to Takata positive sera rendered them negative. This would seem to indicate that this product of the healthy liver inhibits the flocculation and is present in inadequate amounts when the liver is diseased. On the other hand, the experiments of Jurgens²⁶ may be taken as contrary evidence. He found that extirpation of the liver in geese did not produce positive Takata Ara reactions, even though all evidences of fatal liver insufficiency developed. Furthermore, in evaluating any experimental work involving the use of heparin, the fact stressed by Fuchs² should be borne in mind. He called attention to the impurity of the available heparin preparations and cautioned against drawing false conclusions from the results obtained with them. To date, insufficient work has been done to substantiate the claim that heparin is an important factor in the mechanism of the Takata Ara reaction.

The recent work of Oefelein²⁸ opened a new line of investigation into the mechanism of the Takata Ara reaction. Knigge,²⁹ and Rohls and Kohl Egger³⁰ had claimed that the important factor in the reaction was the hydrogen ion concentration and the salt content of the serum. Their results showed that the flocculation was less likely to occur when the medium tested was strongly acid, and that, when the alkaline content was high, flocculation was more pronounced. It had also been shown that the addition of a small amount of ammonium sulfate to the Takata Ara reagent always produced a white precipitate. Accepting these findings and the contention of von Schroeder³¹ and Thannhauser³² that the liver forms urea from the ammonia in the circulating blood, Oefelein concluded that the amount of ammonia ion present in the blood serum would be a measure of the ability of the liver to form urea, the higher the ammonia level the poorer this function. Therefore, apparently with the following in mind: first, that the Takata Ara reaction is a test of liver function; second, that the ammonia level in the blood is an index of one of the functions of the liver, and third, that the Takata Ara reaction is determined by the presence of the ammonia ion, Oefelein studied the ammonia level in the blood serum and its relation to the Takata Ara reaction.

Using the Folin Denis³³ method of determining preformed ammonia, but employing blood serum instead of whole blood, he found that in all cases with a

positive Takata-Ara reaction the ammonia level was greatly increased. He obtained ammonia values of 1.02 to 2.4 mg. per cent where the reaction was distinctly positive; 0.76 to 0.85 mg. per cent in cases with a mildly positive reaction; and 0.2 to 0.58 mg. per cent in all cases in which the reaction was negative. He concluded that there was a definite parallelism between the degree of positivity of the Takata-Ara reaction and the level of the ammonia in the blood serum. Impressed by these striking findings, we set out to determine the ammonia content of the blood serum and the Takata-Ara reaction in a series of cases.

METHOD AND PROCEDURE

Our first problem was to select the best available method of determining the ammonia level in the blood. We made a careful study of the various methods appearing in the literature. This led to the observation that the procedure described by Folin³⁴ in 1932 was the least complicated and the one used by most recent investigators. We chose this method for these reasons and also because it was similar to the one employed by Oefelein in his work. After performing the test many times, we found that the substitution of titration for the colorimetric determination originally described gave results which to us appeared more accurate.*

Our determinations were invariably started within one and one-half hours of the removal of the blood from the vein. Folin³⁵ and others^{36, 37} call attention to the fact that blood on standing rapidly decomposes with the formation of ammonia. Therefore any determination of ammonia in the blood, in order to be accurate, should be done within two or at most three hours. To exercise the greatest possible precaution against alteration of the blood ammonia following venepuncture, we used the method of collecting specimens described by Atchley, Loeb, and others,³⁸ which they employed for the purpose of assuring minimal changes of the pH level in blood serum. The blood was drawn from a cubital vein into a syringe well lubricated with mineral oil and delivered under a two-inch column of oil into a centrifuge tube. This was allowed to stand for exactly one hour, after which the clot was separated with a glass rod and the tube centrifuged. The serum was then drawn off from beneath the mineral oil column and determinations of the ammonia content of the serum and the Takata-Ara reaction were immediately begun. The method of performing the Takata-Ara test and the manner of interpreting the results were the same as those described in an earlier communication.³⁹

The material upon which this report is based is to a large extent drawn from our series in which the clinical significance of the Takata-Ara reaction was studied. This consisted of sera from a large variety of cases. Some of the cases had outspoken cirrhosis of the liver, others had various types of parenchymatous liver disease, and a third group had no obvious involvement of the liver. We

*Oefelein employed the method devised by Folin and Denis³⁴ with the modifications of Thierfelder as described in Hoppe-Seyler and Thierfelder's *Handb. physiol. u. path. chem. analyse* (ed. 9, Berlin, 1934, Julius Springer). We also used methyl red as indicator. Since the quantities of ammonia were so small, we found it preferable to use a weaker acid in the receiving bottle and therefore substituted 0.01 normal sulphuric acid for the 0.1 normal acid. During the process of aerating the serum, a great deal of foaming occurred which could be checked by the addition of a few drops of amyl alcohol.

could, therefore, reasonably anticipate Takata-Ara reactions ranging from strongly positive to negative. The entire series numbered 56 cases.

RESULTS

In order to present the results most clearly, we have in Table II divided our cases into four groups according to the degree of positivity of the Takata-Ara reaction. In each case we have listed the ammonia content of the blood serum. It will be noted that the ammonia level ranged from 0.136 to 0.442 mg. per cent in the cases giving a strongly positive reaction; from 0.170 to 0.680 mg. per cent in those with a positive reaction; and from 0.170 to 0.849 mg. per cent in those giving a weak positive reaction. In the group which showed a negative Takata-Ara reaction, the ammonia level varied from 0.034 to 0.786 mg. per cent.

In a small group, 28 cases, we also studied the relationship of the Takata-Ara reaction to the total proteins and serum albumin and globulin. The results of this study are shown in Table III.

COMMENTS

It can readily be seen from Table II that our tests failed to show any relationship between the level of the ammonia in the blood serum and the degree of positivity of the Takata-Ara reaction. As a matter of fact, some of the highest ammonia figures we obtained were in the group which gave negative reactions. Our findings, therefore, fail to substantiate the claims made by Oefelein. Furthermore, it should be noted that our highest figures are considerably lower than the high readings obtained by this author.

Staub and Jezler criticize Oefelein's assumption that the ammonia radical plays an important rôle in the production of a positive Takata-Ara reaction. They point out the fact that the phenomenon observed when ammonium sulfate is added to Takata-Ara reagent is a precipitation and not a true colloidal flocculation, and hence the result cannot be interpreted as a positive Takata-Ara reaction. In addition, Oefelein's assumption that the ammonia level in the blood is an index of the degree of liver insufficiency is open to question. Although it is known that the liver forms urea from ammonia,⁴⁰ it does not follow that an elevated ammonia level in the blood necessarily points to a deficiency in liver function, since the formation of ammonia is apparently also a property of organs other than the liver,⁴¹ and since ammonia is also absorbed in appreciable amounts from the intestine.⁴² Another point which may be raised in evaluating Oefelein's work is the high ammonia figures reported by him. It is known that "the toxic properties of ammonia preclude the possibility of its existence in appreciable amounts in the circulating blood."⁴³

Although we were aware of the possible objections to Oefelein's line of reasoning on physiologic grounds, we felt that these did not invalidate his experimental findings which so definitely pointed to an elevated ammonia level as a primary factor in the production of a positive Takata-Ara reaction.

It will be seen from Table III that in 13 of our 16 cases, where the reaction was positive, there was an alteration in the albumin-globulin ratio.

TABLE II

RELATIONSHIP OF TAKATA-ARA REACTION TO THE AMMONIA LEVEL IN THE BLOOD SERUM

CASE NO.	DIAGNOSIS	TAKATA-ARA REACTION	NH ₃ IN MG. PER 100 C.C.
3	Laënnec cirrhosis	Strong positive	0.136
10	Syphilitic cirrhosis	Strong positive	0.340
25	Laënnec cirrhosis	Strong positive	0.442
10	Syphilitic cirrhosis	Positive	0.212
14	Senile cataract	Positive	0.170
15	Syphilitic cirrhosis	Positive	0.425
17	Laënnec cirrhosis	Positive	0.680
19	Chronic myelogenous leucemia	Positive	0.272
29	Metastases to peritoneum and omentum	Positive	0.510
38	Lobar pneumonia	Positive	0.374
40	Chronic adnexal disease	Positive	0.612
9	Hepatomegaly of undetermined origin	Weak positive	0.255
12	Acute hepatitis	Weak positive	0.612
13	Arteriosclerotic heart disease (decomp.)	Weak positive	0.340
16	Syphilitic hepatitis	Weak positive	0.170
34	Ca of gall bladder with liver metastases	Weak positive	0.340
37	Arteriosclerotic heart disease (decomp.)	Weak positive	0.442
42	Undiagnosed neoplasm of liver	Weak positive	0.408
44	Ca of ovary, metastases to liver	Weak positive	0.408
48	Adenocarcinoma of pancreas	Weak positive	0.849
56	Acute catarrhal jaundice	Weak positive	0.226
1	Acute catarrhal jaundice	Negative	0.714
2	Nonspecific colitis	Negative	0.068
4	Hypertensive heart disease (decomp.)	Negative	0.306
5	Acute coronary thrombosis	Negative	0.042
6	Arteriosclerotic heart disease (decomp.)	Negative	0.085
8	Hepatomegaly of undetermined origin	Negative	0.238
11	Cyst or tumor of the liver	Negative	0.340
18	Rheumatic heart disease (decomp.)	Negative	0.119
20	Lung abscess	Negative	0.187
21	Hypertensive heart disease (decomp.)	Negative	0.272
22	Desmoid tumor of abdominal wall	Negative	0.272
23	Arteriosclerotic heart disease (decomp.)	Negative	0.642
24	Thrombocytopenic purpura	Negative	0.408
25	Laënnec cirrhosis	Negative	0.334
26	Multiple liver abscesses	Negative	0.340
27	Acute catarrhal jaundice	Negative	0.736
28	Lymphosarcomatosis	Negative	0.786
30	Obstruction in common bile duct (stone)	Negative	0.509
31	Acute follicular tonsillitis	Negative	0.323
32	Gonococcal arthritis and prostatitis	Negative	0.425
33	Bronchopneumonia	Negative	0.255
35	Stone in common bile duct	Negative	0.187
36	Acute catarrhal jaundice	Negative	0.306
39	Arteriosclerotic heart disease	Negative	0.208
41	Thyrototoxic heart disease (decomp.)	Negative	0.374
43	Lobar pneumonia, rheumatic heart disease	Negative	0.442
45	Undiagnosed	Negative	0.340
47	Normal	Negative	0.762
49	Acute upper respiratory infection	Negative	0.544
50	Rheumatic heart disease (inactive)	Negative	0.750
51	Coronary sclerosis	Negative	0.476
52	Acute upper respiratory infection	Negative	0.442
53	Hypertensive heart disease	Negative	0.408
54	Pleural effusion	Negative	0.204
55	Coronary thrombosis	Negative	0.272
57	Bronchopneumonia	Negative	0.452
58	Pleuritis sicca	Negative	0.068

TABLE III
RELATIONSHIP OF THE TAKATA-ARA REACTION TO THE SERUM PROTEINS

CASE NO	TAKATA-ARA REACTION	TOTAL PROTEIN	ALBUMIN	GLOBULIN	ALBUMIN GLOBULIN RATIO
3	Strong positive	3.91	1.08	2.83	0.38
7	Strong positive	3.80	2.31	3.04	0.92
10	Strong positive	3.22	2.44	2.78	0.88
25	Strong positive	3.20	2.95	2.25	1.31
71	Strong positive	6.20	2.14	3.91	0.59
71	Strong positive	4.63	2.21	2.48	0.89
17	Positive	6.25	1.51	2.74	1.28
61	Positive	8.51	4.12	4.11	1.01
67	Positive	.75	2.16	1.59	1.6
68	Positive	4.69	2.12	2.57	0.83
70	Positive	7.20	4.01	3.19	1.26
72	Positive	4.16	1.87	2.59	0.72
74	Positive	4.41	1.87	2.59	0.72
13	Weak positive	6.25	3.12	3.13	1.00
37	Weak positive	6.03	3.41	2.64	1.29
60	Weak positive	6.86	4.02	2.94	1.41
2	Negative	4.69	2.94	1.75	1.68
10	Negative	6.23	3.30	2.95	0.89
21	Negative	5.25	3.00	2.25	1.3
21	Negative	5.83	3.30	2.55	1.29
25	Negative	3.80	2.81	.05	0.92
45	Negative	5.07	2.74	2.33	1.17
70	Negative	5.51	2.81	2.70	1.04
62	Negative	5.22	3.12	2.10	1.48
63	Negative	4.68	2.68	2.00	1.34
64	Negative	6.53	3.75	2.80	1.34
65	Negative	6.25	3.13	3.12	1.00
75	Negative	2.75	1.27	1.48	0.83

garded 13 as the lowest normal relationship of albumin to globulin.) In just 50 per cent of our positive cases was the ratio below 1. These findings are in agreement with those reported by other investigators. However, it must be pointed out that in 7 of the 12 negative reactions there was also an altered ratio, 25 per cent of these negative cases showing a ratio of below 1. In only 5 of the 16 positive cases was the globulin figure above 3. The 11 cases with positive Takata-Ara reactions in which the globulin was below 3 constitute a much larger percentage than that obtained by Kirk. Furthermore, in 2 of the 12 cases with negative reactions the globulin figure was above 3. Skouge, in a large group of noncirrhotics with extremely high globulin figures, obtained negative Takata-Ara reactions.

CONCLUSIONS

1. There is no relation between the ammonia content of blood serum and the Takata-Ara reaction.
2. In most cases with a positive Takata-Ara reaction there is an alteration of the albumin globulin ratio.
3. The altered albumin globulin ratio, although not the sole factor, is probably the most important one in the mechanism of the Takata-Ara reaction.

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ACTIVE IMMUNIZATION AGAINST TETANUS BY MEANS OF TETANUS TOXOID ALUM PRECIPITATED REFINED

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BERGEY and Etris,¹ Jones and Moss,² Gold,³ Hall,⁴ McBryde, and Cowles⁵ have demonstrated that the injection into human beings of two doses of alum precipitated tetanus toxoid is followed by the appearance in the blood of appreciable quantities of tetanus antitoxin. It has also been established that these injections can be given with safety and without the development of unpleasant reactions. The amount of tetanus antitoxin produced, and its persistence in the blood appear to be very variable. These two factors are not dependent upon race, sex, or age but seem to be related to the individual constitution and the state of health of the host at the time of antigenic stimulation.

An analysis of the published data reveals several important questions which must be answered before active immunization against tetanus can be recommended as a routine procedure. 1. What is the minimum titer of tetanus antitoxin that will protect an actively immunized individual against infection with *Ct tetani*? 2. What is the optimum dose of toxoid to be injected? 3. What interval of time should elapse between the two injections to obtain the best antitoxin response? 4. How soon after the course of immunization is completed does immunity develop? 5. How long does active immunity last? 6. If such an immunity is lost, how soon can the antitoxin titer be restored to a protective level following the injection of a third or subsequent doses of alum toxoid?

In reference to the first question, two avenues of experimental approach exist: (1) protection tests in actively immunized laboratory animals, and (2) studies of antitoxin titers in passively immunized human beings. Although Sneath and his associates⁹ submit that an actively induced resistance to infection by *Cl. tetani* as shown in guinea pigs is the most convincing criterion possible of attainment in the laboratory, one must always keep in mind the possibility that such findings are not directly transferable to man. Moreover, although the infective experimental dose is determinable, the amount of free toxin produced by the spores in the wound and responsible for the clinical picture of tetanus, is a variable factor beyond control.

Jones and Jamieson¹⁰ found that guinea pigs that had received one or more injections of tetanus alum-precipitated toxoid and were injected within two months, were protected against a massive dose of tetanus spores which killed normal pigs in from eighty-eight to one hundred and twelve hours. With the infective dose used, these workers found that it required from two to three units of antitoxin per cubic centimeter of blood in the immunized pig to neutralize the toxin produced from the infection. In this regard, it is interesting to note that though some of the guinea pigs had as high as four units of antitoxin, they developed localized tetanus from which, however, they completely recovered. These workers also found that a massive dose of tetanus spores did not markedly accelerate the production of antitoxin in guinea pigs that had been previously immunized with alum tetanus toxoid. This observation destroys the hope entertained by some⁷ concerning the possible accelerating effect to be exerted upon the antitoxin level of the blood of an immunized person by tetanus spores if present in a wound of such a subject.

Sneath, Kerslake, and Scruby⁹ injected various dilutions of spore suspensions mixed with equal volumes of 50 per cent calcium chloride intramuscularly into normal animals in doses of 0.1 c.c. They found that more uniform results may be expected when the spore distribution in the test dose is of the order shown by dilution 10^{-11} . The computable number of spores, varying from 3 to 50 per dose, when combined with calcium chloride, induced tetanus and killed unprotected guinea pigs without an experimental constant interval of forty-eight to seventy-two hours. Upon the injection of this lethal spore dose into a group of 55 guinea pigs actively immunized by means of tetanus toxoid, they found that 80 per cent of the animals were completely protected, 11 per cent were partially protected, and 9 per cent died of tetanus. In a similar group of guinea pigs that received 1500 units of antitoxin intraperitoneally, immediately after injection of the lethal spore dose, 24 per cent were completely protected, 38 per cent were partially protected, and 38 per cent died of tetanus. From their studies on the actively immunized group, it would appear that in the guinea pig, the level of 0.01 unit of tetanus antitoxin per cubic centimeter of serum is a critical level in tetanus immunization. Above that level, in 45 animals 13 per cent showed signs of tetanus, from which they recovered, while the remaining 87 per cent were completely protected. Sneath and his co-workers could not explain why three animals, showing less than 0.01 unit, were com-

pletely protected, while seven showing antitoxin in excess of that level (two having more than 0.1 unit) were only partially protected. They feel that in view of these discrepancies, it may be reasonable to suggest the possibility that antitoxin per se is not the sole factor influencing protection against the lethal spore dose. They conclude that since infection with *C. tetani* is a localized process, it is probable that such an antitoxic level (0.01 unit) as will prevent the manifestations of tetanus in guinea pigs would also be sufficient to prevent the disease in man. The incidence of localized tetanus in 13 per cent of the immunized animals is, however, worthy of emphasis, since in the human being, one must strive for complete protection in all cases.

Cowles⁸ carried out a similar type of experiment on actively immunized guinea pigs and mice. Upon injection of 100,000 tetanus spores and 10 mg of calcium chloride contained in a 0.2 cc volume, into a group of 27 immunized pigs, he found them completely protected against the disease during a three months' follow up period while 26 out of the 28 controls died in less than forty six hours with tetanus. Before injection 1 animal had less than 0.2 unit of antitoxin, 24 had 0.20 to 1 unit and 2 had more than 1 unit. A second group of immunized animals was similarly treated except that the infective dose contained 25,000 tetanus spores. In this group Cowles encountered the same apparent inconsistency noticed by Sneath in his experiments. Two pigs that had 0.10 unit of antitoxin per cubic centimeter and a third animal that showed 1 unit per cubic centimeter died of tetanus in from six to eleven days, while 2 animals that had 0.05 unit and 1 guinea pig that had 0.02 unit were completely protected against the disease. In a group of 8 mice whose antitoxin titer ranged between less than 0.05 to more than 0.05 unit of antitoxin per cubic centimeter, 5 died of tetanus in from three to seven days, 2 were paralyzed but survived, and 1 was completely protected when injected with 5,000 tetanus spores and 5 mg of calcium chloride in a 0.1 cc volume. The controls were all dead in forty eight hours. A group of mice showing 0.10 unit or more were completely protected against an infection of 100,000 spores. From his animal experiments, Cowles concludes that although it is probably impractical to define the minimum titer which will assuredly protect against tetanus, antitoxin values of 0.10 or 0.20 unit per cubic centimeter of serum can give a fairly certain protection in immunized guinea pigs and mice at the time of infection. He feels that though 0.10 unit is not sufficiently great to protect all animals against a maximum infection, it is probably much larger than is necessary to care for many infections resulting from wounds judged to be too slight for surgical attention, and probably large enough to care for the majority of injuries which receive surgical treatment. He asserts that pending the acquisition of more information, conservative opinion may demand the maintenance of such a titer in cases where much reliance is to be placed on the immunity.

Bergey and Citrus^{1, 2} have suggested drawing an inconclusive analogy with diphtheria, that 0.01 unit may be satisfactory for protection. In a later publication¹¹ the same authors maintain that 0.01 unit is "the least amount of antitoxin necessary for protection and is based on the finding in the serum of

TABLE I
GROUP A

TETANUS TOXOID, ALBUM PRECIPITATED, REFINED No. 76812-1

Two doses, 0.5 c.c. each, given 645 days apart.

Titer expressed in units of tetanus antitoxin per cubic centimeter of blood serum.

CASE	CONTROL TITER	DAYS AFTER FIRST DOSE		DAYS AFTER SECOND DOSE									
		TITER		TITER									
6611	0.003	24 days 0.003	645 days +0.003 -0.01	34 days +0.25	90 days +0.25	184 days +0.25	284 days +0.25	365 days +0.25	455 days +3.0 -5.0	578 days 3.0	679 days 2.0		
6617	0.003	24 days 0.003	645 days 0.003	32 days +0.25	89 days +0.25	200 days +0.10 -0.25	285 days 0.25	365 days +0.10 -0.25	455 days 0.25	687 days 0.10			
6644	-0.003	26 days -0.003	644 days -0.003	32 days +0.25	90 days +0.25	184 days 0.10	284 days +0.01 -0.10	290 days +0.01					
6657	+0.003	23 days +0.01 -0.10	644 days 0.003	32 days +0.10	90 days +0.25	184 days 0.10	284 days +0.01 -0.10	284 days +0.01					
6645	-0.003	23 days -0.003	644 days 0.003	32 days +0.25	90 days +0.10 -0.25	184 days +0.01 -0.10	284 days +0.01 -0.10	284 days +0.01 -0.10					
6649	-0.003	26 days -0.003	644 days -0.003		90 days 0.10	185 days +0.01 -0.10	284 days +0.01 -0.10						
6643	-0.003	23 days -0.003	644 days -0.003	33 days 0.25	89 days +0.10 -0.25	184 days 0.01							
6642	-0.003	23 days -0.003	644 days -0.003	32 days +0.10 -0.25	90 days +0.01 -0.1	184 days 0.01	284 days 0.01						
6614	+0.01 -0.10	24 days +0.01 -0.10	645 days +0.01 -0.10	32 days 0.25	90 days +0.01 -0.10	172 days +0.01 -0.10	284 days -0.10						
6618	-0.003	24 days -0.003	645 days -0.003	32 days 0.10		171 days +0.01 -0.10	318 days -0.01	529 days +0.01 -0.1					
6630	0.003	24 days -0.003	645 days -0.003	32 days 0.25									

* More than
- Less than

the person who showed least response to the antigenic effect of the toxoid one year after the second dose. This individual had 0.01 of a unit of tetanus antitoxin." This finding cannot, however, be accepted as indicating the protection level against infection.

Our studies on passive immunization confirm Cowles' experimental findings. Prophylactic passive immunization with 1500 units of tetanus antitoxin has been successfully used to prevent lockjaw following injuries in both war and civil life. Titrations following the injection of such a prophylactic dose⁴ reveal the presence of 0.1 to 0.25 units of antitoxin per cubic centimeter of blood serum. Hence, to be of value, active immunization must at least produce 0.1 unit of antitoxin per cubic centimeter. This minimal protective value does not appear to us to be too high, since we have on several occasions encountered control values of more than 0.01 unit of antitoxin in persons who were never immunized passively or actively, and who would consequently be considered susceptible to tetanus. Until direct proof exists that a lower antitoxin level is sufficient to prevent tetanus, one must insist on the presence of 0.1 unit of antitoxin per cubic centimeter of blood serum in order to consider an actively immunized person protected against this disease.

From our experience with human beings, we feel that a 1 cc dose of tetanus toxoid is more effective than smaller quantities of this antigen. We have also found⁴ that when the two doses are given close together (one month or less), the antitoxin titer that follows is not as high as when they are injected at an interval of ninety days. Berger and Etris¹² had a similar experience. McBryde injected the two doses seventy-three days apart. Hall⁶ allowed six weeks to elapse between the two injections. Jones and Moss³ administered the two doses four weeks apart. Cowles⁴ allowed an interval of eleven weeks between the two doses.

In most individuals the first injection of toxoid serves to prepare the host so that after a suitable interval of time, the administration of the second dose is followed by a rather prompt appearance in the blood of an increased amount of tetanus antitoxin. We have stated that this interval should preferably be three months. Actually, how long can this interval be?

Fifty adult workers (group A) were immunized with two 0.5 cc doses of Mulford tetanus toxoid alum precipitated refined lot No. 76812-1, given six hundred and forty-five days apart. Six weeks after a single injection of 0.2 cc, 1.0 cc, and 2.0 cc of this toxoid was given to three groups of guinea pigs respectively, then blood serums were tested according to the standard procedure of the National Institute of Health. The pooled serums of the 0.2 cc group showed 5 units of antitoxin. The 1 cc group showed 2 units, while the guinea pigs injected with 2 cc of this antigen had an antitoxin content of 3 units. These tests should not be interpreted as indicating that the smaller amount of tetanus toxoid constitutes an optimum dose.

The follow up results on 11 members of this group A are given in Table I. Case 6614 is interesting because his control titer was greater than 0.01 unit per cubic centimeter. He had never been passively immunized, and the values ob-

TABLE III
GROUP C
ANTITOXIN RESPONSE AFTER THE INJECTION OF A THIRD DOSE OF TETANUS TOXOID, ALUM PRECIPITATED, REFINED
Titer expressed in units of tetanus antitoxin per cubic centimeter of blood serum.

CASE	NO. OF DAYS BETWEEN SECOND AND THIRD DOSES	CONTROL TITER	THIRD DOSE No. 5969-1	DAYS AFTER THE THIRD DOSE TITER										
				4	7	9	14	28	33	92	139	182	212	273
6642	308	+0.01 -0.10	0.5 c.c.		0.10		+0.25	+0.25		+5.0		+0.25		+0.10 -0.25
6644	308	+0.01 -0.10	0.5 c.c.		0.25		+0.25	+0.25		+0.25		+0.50 -0.50		0.25
6645	308	+0.01 -0.10	0.5 c.c.		+0.10		+0.25	+0.25		+0.25		+0.10 -0.25		0.10
6714	230	+0.01 -0.10	0.5 c.c.		+0.25		+0.25	+0.25		0.10	0.10			+0.10 -0.25
6614	308	-0.10	1.0 c.c.		-0.10		+0.25	0.25		-0.10		0.01		0.01
6619	308	+0.01 -0.10	1.0 c.c.		0.25		+0.25	+0.25		+0.25		0.10		+0.01 -0.10
6657	308	+0.01 -0.10	1.0 c.c.			+0.25		+0.25						-0.10
6613	200	0.01	0.5 c.c. No. 76812-1	+0.01 -0.10	+0.10 -0.25	+0.25			0.25		0.10	-0.10	+0.01 -0.10	

again on the seventh day, there was noted a very marked rise in the antitoxin content of their blood, reaching from 1 to 25 to 50 units. From an average control titer of 0.032 unit, there was an increase to an average titer of 9.6 units of tetanus antitoxin.

Cowles⁸ administered a third dose of alum toxoid to 10 subjects, thirty-nine weeks after the second injection. No increase in titer was noted until the fourth day after the repeat injection, when 1 out of 6 subjects tested showed 0.1 unit. On the fifth day, 4 subjects had +0.1 unit, while 3 still showed 0.05 unit or less. On the seventh day, 3 cases had 0.50 unit, 1 showed 5 units, and 1 still showed 0.05 unit. The latter when tested twenty-four hours later showed a titer of 0.20 unit. On the tenth day, 1 subject showed 0.50 unit while another one had 10 units. The latter on follow-up tests revealed a decided drop in the antitoxin content of his blood, showing 5 units on the fortieth day and 2 units one hundred and forty days after the repeat injection. Cowles concluded that the response to a subsequent injection is not significant until the fourth or fifth day to prevent tetanus, but did not know whether or not this was soon enough. Although the influence of such a delayed rise in titer cannot be determined without further study, Cowles felt that upon injury, the repeat injection of toxoid should certainly be made if antitoxin is to be withheld.

Seven subjects (group C, Table III), members of our groups A and B, received a third dose of tetanus alum-precipitated toxoid No. 5969-1, two hundred to three hundred and eight days after the second injection. This lot of toxoid was freshly prepared from a toxin containing over 20,000 M.L.D. The injection of 0.5 c.c. of this toxoid into a group of standard guinea pigs produced 5.0 units of tetanus antitoxin per cubic centimeter of blood serum at the end of six weeks. Similar values were produced following the injection of 1.0 c.c. of this antigen. The eighth member of group C was injected with the aged toxoid No. 76812-1, used for the basic course of immunization. Their serums showed -0.1 unit before injection of the repeat dose. All but three subjects got 0.5 c.c. of the toxoid; the rest received 1.0 c.c.

Case 6643, whose control titer was 0.01 unit, showed +0.01 -0.10 unit when tested four days after the third dose. On the seventh day, all patients but one showed 0.1 unit or more. The exception when tested again a week later showed +0.25 unit. Case 6614 dropped to -0.10 unit when tested three months after the repeat injection. The rest still showed good protective values. Six months after the third dose another subject dropped below 0.1 unit. Three months later, a third patient showed -0.1 unit. When tested one year after the repeat injection, over half of the members of the group had dropped below the minimum protective level of antitoxin.

Upon comparison of the type of response obtained after the third dose of toxoid with that obtained following the injection of the second dose, one finds that in four persons, the third dose was followed by a more prolonged maintenance of the protective titer. In two, the duration of the protection was identical. As in the case of the second dose, the response following the injection of a repeat dose of toxoid is variable and in some subjects may be short-lived. Hence a false sense of security may result. A definite period of time elapses

before a protective titer develops following the repeat injection. In some cases, it may be longer than a week. The question naturally arises as to whether tetanus infection may not develop before the antitoxin titer has a chance to reach a protective level. Additional data should be collected before a definite conclusion can be reached on this point. Meanwhile, it is important to insist on conservative standards of protection.

It may be that antitoxin per se is not the sole factor concerned in the process of immunity against tetanus. Both Sneath and Cowles present suggestive experimental evidence in support of this view. In an addendum to his report, Cowles cites the case of a subject who had recovered from tetanus eight years before the injection of 0.5 e.e. of alum toxoid, and who reacted to this first injection in the same manner as normal individuals do. This would indicate that the toxin concerned in the attack of tetanus had not, in this case, constituted an antigenic stimulus, since a state of "sensitization" did not result.

Similarly Case 6645 of our group A had also recovered from tetanus in 1923. Ten years later his antitoxin titer was found to be -0.003 unit. There was no appreciable increase in the antitoxin content of his blood following the first injection of alum toxoid, indicating that the tetanus infection from which he had recovered had not affected his immunity mechanism in the same fashion as the injection of the first dose of toxoid. The response of this subject to subsequent injections of toxoid was similar to that of normal individuals.

Case 6643 received a fourth dose of alum toxoid (0.5 e.e.) two hundred and twelve days after the third injection, when the antitoxin content of his blood was found to be +0.01 -0.10 unit. Seven days later, his titer was +0.25 unit. When tested again four months later, he showed 0.25 unit. One year after the injection of the fourth dose of toxoid, the antitoxin content of his blood was found to be +0.1 unit.

COMMENT

The most important question requiring further investigation is the determination of the actual amount of antitoxin necessary to protect an individual against tetanus. More experimental data and perhaps field work, possibly in war zones, rigidly controlled to satisfy statistical requirements are needed. Around it hinge the limitations of active immunization against tetanus as brought out by this study. At present active immunization may prove to be of value in military service and in certain phases of civil life where injuries occur repeatedly. It will also prevent the occurrence of "delayed" or so-called "chronic tetanus."

CONCLUSIONS

1. A protective antitoxin titer of 0.1 unit or more develops in five to fourteen days following the injection of a second dose of alum toxoid, even when the latter is given two years after the first dose.

2. The duration of the protective level of antitoxin varies a great deal. It may disappear within ninety days after the second injection or it may last over two years.

3 The antitoxin titer can be raised to a protective level by the injection of a third or subsequent doses of alum toxoid

4 From five to more than seven days elapse after the "repeat" injection of toxoid before there appears 0.1 unit of antitoxin in the blood of actively immunized persons

5 The immunity that develops following the injection of a "repeat" dose of alum toxoid is also variable in duration. It may drop below 0.1 unit in three to six months after the third or subsequent injections

6 If an injury occurs during the interval of time that elapses before the basic course of immunization is completed and within a few days after the second or subsequent injections of alum toxoid when the antitoxin level of the blood has not as yet reached 0.1 unit it may be necessary to resort to passive immunization in order to insure full protection against tetanus

The antitoxin titrations reported in this paper were carried out at the Mulford Biological Laboratories. Sharp and Dohme who kindly supplied me with the necessary material for immunization. I also wish to thank the Stickville Mills Co. Wallingford Pa. for their cooperation in securing their personnel to serve as subjects for this investigation

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ELECTROCARDIOGRAPHIC CHANGES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF MAGNESIUM SULFATE

AN EXPERIMENTAL STUDY ON DOGS*

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THE present study was undertaken to determine the effect upon the conductive system, as measured by the electrocardiogram, of disturbances in the chemical balance ordinarily maintained by the body. Alteration in the magnesium content constituted the first portion of this study. Sudden death following the injection of a magnesium salt for treatment of epilepsy and other conditions is not an uncommon occurrence. The mechanism of such untoward results, however, has not been adequately described.

Jolyet and Cahours¹ showed that the intravenous injection of a solution of magnesium salts caused loss of motility of voluntary muscles, and Matthew Hay² demonstrated that respirations ceased following large doses, although the heart continued to beat slowly. Larger doses produced a sudden cardiac standstill during diastole. Matthews and Brooks,³ in a series of experiments on dogs, concluded that the action of magnesium sulfate upon the heart is essentially a depression of the cardiac nervous mechanism, resulting in loss of tone, but that it retains its irritability and that a sufficiently strong stimulus will result in contraction.

Tedesco⁴ made observations of the effect of magnesium chloride upon the electrocardiogram. He concluded that it produced a positive chronotropic effect on the action of the heart, and that the speed of propagation of the stimulus from the auricle to the ventricle was appreciably diminished. He was unable to draw conclusions concerning its effect upon the bundle of His, but was of the opinion that magnesium had a stimulating effect upon the electrical condition of the ventricles.

Zwillinger⁵ observed the effect in man of the intravenous and intracardiac injection of magnesium sulfate when arrhythmias were present. He concluded that it plays a rôle in combating certain forms of paroxysmal tachycardia and extrasystoles. He was able to show experimentally that magnesium sulfate had a beneficial effect in combating digitalis intoxication.

METHODS

In all experiments the dog was placed on the left side. No anesthetic was used. Electrodes were attached to the right front and left rear legs, thus corresponding to the standard second lead. Electrocardiograms were made on

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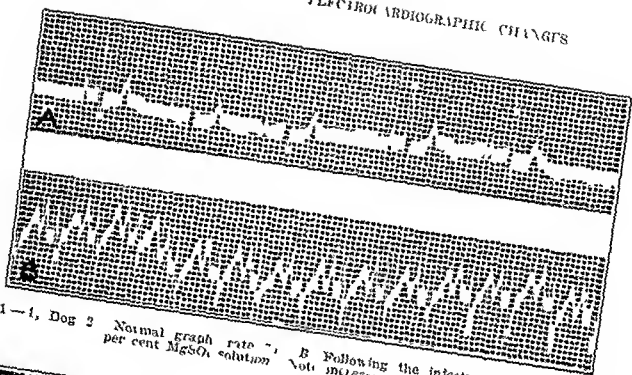


Fig 1—1, Dog 2 Normal graph rate *A*; 20 per cent $MgSO_4$ solution *B* Following the injection of 4 cc of a 20 per cent $MgSO_4$ solution. Note increase in rate (190)

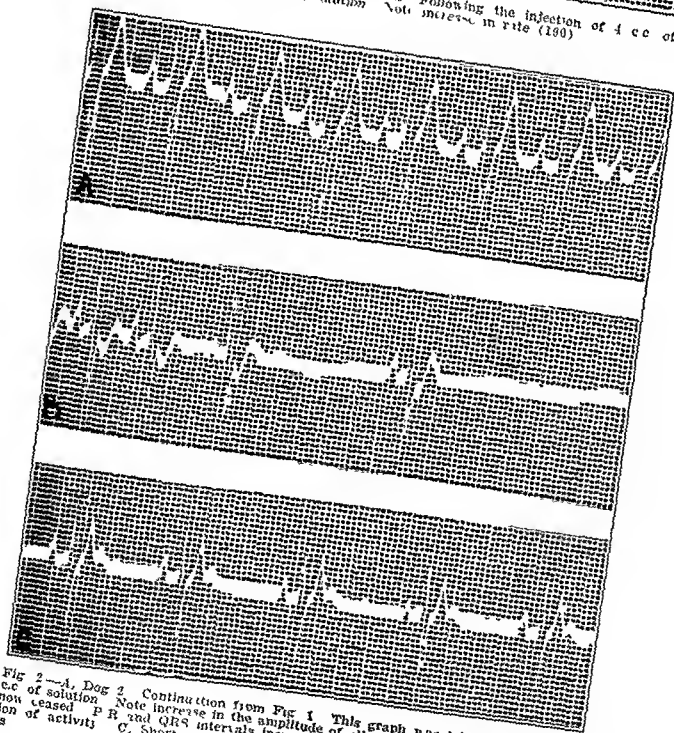


Fig 2—A, Dog 2 Continuation from Fig 1. This graph was taken following injection of 22 cc of solution. Note increase in the amplitude of all complexes. Rate 110. Respiration have now ceased. P R and QRS intervals increased 0.06 second. B Two minutes later with cessation of activity. C, Short resumption of activity after one minute fasting only thirty seconds

a string galvanometer type machine. The magnesium salt was injected into the right saphenous vein. Electrocardiograms were made during injection and at varying intervals from two minutes to two days, as described hereafter.

RESULTS

Experiment 1.—Dog 1, weight 10 kg.; Dog 2, weight 13.1 kg. Following injection of 10 c.c. of 20 per cent magnesium sulfate solution, there was an acceleration of rate from 75 to 90. This coincided with a marked though transient increase in respiration. Otherwise the only other remarkable change was an increased excursion of the T-wave.

The solution was then injected at the rate of about 2 c.c. per minute in Dog 1, and 1 c.c. per minute in Dog 2, and graphs taken at two-minute intervals.

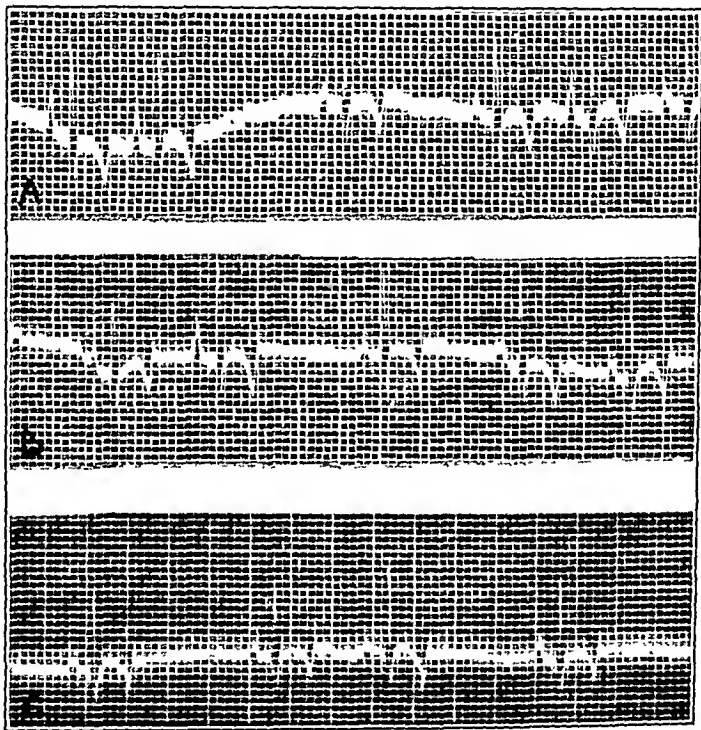


Fig. 3.—A, Dog 3. Normal graph. B, Ten minutes after injection of "therapeutic dose" of $MgSO_4$, P-R interval prolonged 0.02 second. C, One hour later. Return to normal.

The rate increased markedly until in each instance 18 c.c. had been injected. Thereafter it fell slowly. At 28 c.c. respirations ceased, and the heart rate had fallen to 70. Two minutes later the heart suddenly stopped contracting.

As the rate increased (Figs. 1 and 2) there was also an increase in the amplitude of all complexes with a prolongation of both auricular and ventricular conduction time. After a short period of inactivity, the heart resumed a sinus rhythm, although there was marked distortion of the various complexes. In Dog 1, after a second period of complete inactivity, the heart was reactivated following injection of calcium gluconate, and when cessation of activity again occurred, artificial respiration was followed by a rhythm of ventricular origin which lasted for a short period.

Experiment 2—Dog 3, weight 9 kg In this animal the effect of an intravenous injection of magnesium sulfate, in dosage proportionate to the amount sometimes given therapeutically, was noted (22 mg. per kg) Five minutes after administration, the rate had fallen from 100 to 80, the P-R interval was increased (0.02 second), and the T-wave, originally negative, became more so by 25 per cent There was no other discernible difference These changes were present ten minutes after injection but had entirely disappeared at the end of an hour (Fig. 3)

A graph made twenty-four hours later was identical with one made at the beginning of the experiment

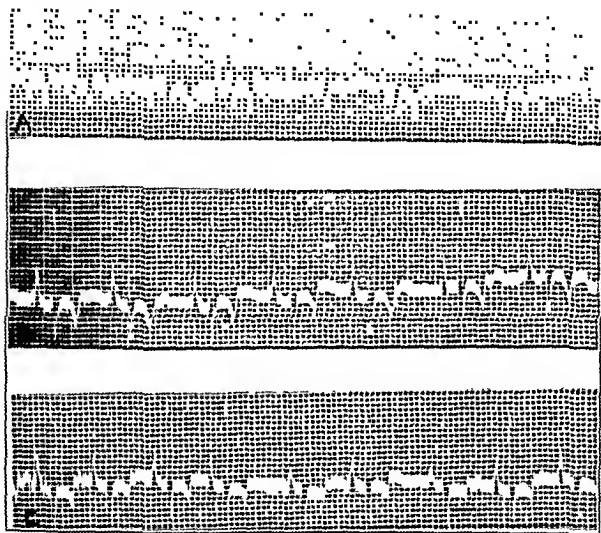


Fig. 4—1 Dog 3 Normal graph This graph was taken twenty-four hours after those in Fig. 3 B, thirty-five minutes after injection of 9 cc of a 20 per cent $MgSO_4$ solution in divided doses Note increased amplitude of all complexes with prolongation of both auricular and ventricular conduction time C Eighty-five minutes later Some effect still evident

Experiment 3 was carried out essentially as Experiment 1, except after injection of 9 cc. of 20 per cent solution of magnesium sulfate, at which point pronounced changes had occurred, the administration of the drug was stopped and the dog allowed to recover Increased excursion of the P- and R waves as well as an increased auriculoventricular conduction time persisted at the end of eighty-five minutes, indicating that in such doses the effect is not a transient one (Fig. 4).

Experiment 4—As magnesium sulfate had been used in the preceding procedures it was decided to use another salt of magnesium to determine whether the latter substance was solely responsible for the effects noted.

Consequently magnesium ehloride was given in an amount containing an equivalent weight of magnesium as employed in the first experiment. The findings were essentially the same as resulted from the use of the sulfate.

CONCLUSIONS

Electrocardiographic studies indicate that magnesium, when injected intravenously, has a definite effect upon the cardiac conductive system.

This effect consists of an early acceleration, followed by a slowing with delay in auriculoventricular and ventricular conduction time and increased excursion of all complexes. This effect may persist for more than an hour after injection of the salt, but is not permanent.

Respirations cease before cardiac activity stops. When the latter occurs, it does so suddenly with a preceding bradycardia, but without any arrhythmia. The heart may spontaneously begin to beat, even after complete cessation of activity.

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THE INCIDENCE OF POSITIVE IMMUNOLOGIC REACTIONS FOR UNDULANT FEVER*

STUDIES OF 5,000 BLOOD SERUM AND 491 SKIN TESTS

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WITHIN the past ten years the medical profession has become increasingly aware of undulant fever, a disease formerly considered as uncommon or rare. A larger number of acute cases of undulant fever are now being recognized because physicians are considering the possibility of the clinical entity in febrile conditions which are not otherwise explained by the physical findings.

It is apparent, however, that few physicians realize the great importance and the high incidence of the chronic and ambulatory forms of the disease which so often cause only vague aches and indefinite complaints. In the past, and even now, patients with these indefinite symptoms have often been dismissed with a presumptive diagnosis of "chronic fatigue" or "neurasthenia."

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The final diagnosis of undulant fever in both the acute and chronic forms depends on laboratory data and includes bacteriologic and immunologic tests. In the chronic form particularly, the symptoms and physical findings are so variable as to make very questionable a diagnosis on a purely clinical basis. Due to difficulty in isolating the causative organism in chronic cases, immunologic procedures, such as the agglutination test and the intradermal test, must be employed to make a definite diagnosis.

Epidemiologic studies, based on laboratory procedures, have been undertaken in various parts of the world to determine the incidence of human brucella infections. The results have been astounding because of the large number of cases, particularly of the chronic type, which have been discovered. The literature on undulant fever is voluminous and references will be found in many of the articles on the subject.

This report covers work which has been carried out to determine the incidence of positive immunologic evidences of undulant fever in unselected patients admitted to a general hospital and its out patient department.

In this study 5,000 consecutive specimens of blood submitted for the Wassermann test by the hospital and its out patient department were examined for brucella agglutinin by the macroscopic tube method. The antigen used for the agglutination test was made from a strain of *B. melitensis* var. *abortus*, recovered from a human case of acute undulant fever. The organisms were grown on a 2 per cent nutrient agar medium for forty eight hours, and then suspended in normal saline solution containing 0.5 per cent phenol. The suspension was kept in the icebox for forty eight hours and at the end of this time proved to be sterile. This was diluted to a turbidity of 300 (silica standard) with 0.5 per cent phenolized normal saline solution. For the test the serum was placed in small test tubes and antigen was added to make two final dilutions of the serum 1:25 and 1:100. The tubes were incubated in a water bath at 54° C for four hours then placed in the icebox overnight and observed the following morning. They were returned to the icebox for twenty four hours longer and the readings made again.

Of the 5,000 blood serums examined routinely 51 showed an agglutination titer of 1:25 and 9 had a positive agglutination reaction in both the 1:25 and 1:100 dilutions. In all 60 patients had positive agglutination reactions. Follow up studies revealed that in 39 of this group on whom positive reactions had been obtained, 24 (62 per cent) were then living on farms. Thirty-one (79 per cent) of the 39 stated that they had drunk mostly raw milk in the past two years, while only eight (21 per cent) drank little or no raw milk during this time.

In this series of 5,000 blood serums, 1,900 (38 per cent) were from men and 3,100 (62 per cent) were from women. Of the 60 positive serums, 21 (35 per cent) were from men and 39 (65 per cent) were from women. This makes an almost equal incidence of positive agglutination reactions among the men and women of this series.

At the time the agglutination tests were being carried out, intradermal tests were also done on the hospital patients (except those seriously ill and

infants less than six months of age). A definite majority of these hospital patients had been living on farms or in farming districts.

The antigen used for the intradermal tests was the same as that for the agglutination tests, except that it was diluted ten times with sterile saline solution containing 0.5 per cent phenol, then heated in a water bath at 60° C. for one hour, and tested for sterility. One-tenth cubic centimeter of this antigen was injected intradermally in the ventral surface of the forearm of the patients.

Local reactions, which occurred during the first twenty-four hours after injection, were disregarded. Mild general reactions occurred in some of the patients in twenty-four to thirty hours. These later showed positive intradermal reactions. In the positive reactors there developed at the site of the injection, in about forty-eight hours, a tender edematous area with central induration, varying from 1 to 2 cm. in diameter, and a peripheral zone of erythema, varying from 2 to 8 cm. in diameter, depending on the severity of the reaction. Erythema alone, without induration, was regarded as a negative reaction. Table I shows the basis for grading the reactions, which was the diameter of the area of induration and of erythema.

TABLE I
GRADING OF SKIN REACTIONS

INTERPRETATION	GRADE OF REACTION	DIAMETER OF INDURATION (CM.)	DIAMETER OF ERYTHEMA (CM.)
Negative	0	0	0
Positive	1	0	1 or more
	2	1	3
	3	1 to 2	4 or more without ulceration
	4	1 to 2	4 or more, with ulceration (in seven to ten days)

In some of the more severe reactions there were reddish streaks extending a short distance up the forearm, suggestive of a lymphangitis. The reaction was usually at its height in from seventy-two to ninety-six hours, and gradually subsided during a period of a few weeks to three months. In some of the more severe reactions, at the end of a week to ten days, the central area softened and then ulcerated with the discharge of thick leucocytic material which did not show bacteria in either smears or cultures.

TABLE II
RESULTS OF INTRADERMAL TESTS ON 491 HOSPITAL PATIENTS

HOSPITAL WARD	NUMBER OF TESTS DONE	NUMBER OF POSITIVE REACTORS	PERCENTAGE OF POSITIVE REACTIONS
Men's Medicine	90	18	20.0
Men's Surgery	83	7	8.4
Total Men	173	25	14.4
Women's Medicine	78	11	14.1
Women's Surgery	91	9	9.9
Obstetrical	87	12	13.8
Total Women	256	32	12.5
Children's	62	3	4.8
Grand Total	491	60	12.2

Table II shows the results of 491 intradermal tests made on patients in the different hospital wards. The patients in the men's medical ward yielded the highest per cent of positive intradermal reactions, while the lowest per cent occurred among the pediatric patients.

A positive agglutination reaction for undulant fever is generally considered as indicating either present or past infection with brucella organisms. The intradermal reaction is believed to have the same significance, but it often is present in the absence of an agglutination reaction. In this series, no patients were found who gave a positive agglutination reaction and a negative intradermal reaction. However, many patients with a negative agglutination reaction gave a positive intradermal reaction. The intradermal test yielded a much higher per cent of positive reactions (12.2) than did the agglutination test (1.2).

Authorities are well agreed that the ingestion of raw milk is a common source of undulant fever. It is interesting to note that of the 60 patients who had positive skin reactions, 55 (91.7 per cent) gave a history of drinking mostly raw cow's milk, one (1.7 per cent) drank raw goat's milk, two (3.3 per cent) stated that they drank only pasteurized milk, and two (3.3 per cent) consumed only canned milk during the past two years. Of the 39 patients showing positive agglutination reactions on whom a milk history was obtained, 31 (72 per cent) drank mostly raw milk during the past two years. It is apparent from these figures that there seems to be a definite direct relationship between the use of raw milk and the occurrence of positive agglutination and skin reactions for undulant fever.

CONCLUSIONS

Five thousand samples of blood have been examined and 491 intradermal tests have been carried out to determine the incidence of positive immunologic reactions for undulant fever.

Sixty (1.2 per cent) of the 5 000 blood samples yielded positive agglutination reactions. Sixty (12.2 per cent) of 491 patients showed positive intradermal reactions.

The incidence of positive immunologic reactions is approximately the same for the men and women in this series.

Patients on the men's medical ward showed the highest incidence of positive intradermal reactions, while the children in this series had the lowest.

There seems to be a direct relationship between the occurrence of positive immunologic reactions and the ingestion of raw milk.

Brucella infections appear to be relatively common, as evidenced by immunologic tests carried out on patients admitted to a general hospital and its outpatient department.

THE CLINICAL UTILIZATION OF BLOOD STUDIES*

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IT IS common knowledge that the practice of medicine is not a fixed but a changing art. The concepts of today are so continually modified by the studies of tomorrow that the theories of today are sometimes the facts of tomorrow, just as the facts of yesterday may become the archaisms of today.

It is this continual realignment of facts, together with the equally constant readjustment of fact and theory, which forces the conscientious physician to continued study.

Perhaps among the more startling, and at times even disconcerting, phases of early days in practice is an appreciation of the dearth of the typical textbook case. In contrast to the clear-cut, definite entities of the lecture hall and clinic, one seems to see rather diagnostic problems bristling with complexities, each of which constitutes in essence an examination of one's knowledge in its entirety. These experiences, if utilized and digested, lead inevitably to the realization that the physician must acquire and develop an investigative viewpoint. It is not enough to give the disease a name. Its nature, its causative factor or factors, and the mechanism whereby the effects leading us to suspect its presence are manifested must all be appreciated and understood if attempts to control it are to be intelligently conceived and wisely directed.

To this end, fortunately, the physician of today may call to his aid innumerable refinements applicable to the examination of the patient, among them the numerous resources of the clinical laboratory.

So many and so great have been the advances in the field of laboratory medicine that there has been at times some indication of unfortunate tendency to utilize them to the neglect of the six most valuable assets in the study of any patient or any disease, to wit: one's five senses, carefully and thoroughly trained for the work at hand, and, perhaps most valuable of all—common sense in their utilization and direction.

The laboratory is not a diagnostic slot machine. It constitutes primarily and essentially simply *a phase in the examination of the patient*. It must be used as an adjunct to the ordinary methods employed in the study of the patient and disease and as such cannot safely nor profitably be neglected. And just as the clinical pathologist must be a physician with sufficient clinical training to enable him to interpret correctly the data accruing from his examinations, so the physician must be sufficiently a clinical pathologist to select from the multitude of those available the particular procedures most likely to be informative in the particular problem at hand.

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Just as Lord Moynihan is said to have regarded every surgical operation as an experiment, and Sir William Jenner to have looked upon the administration of a drug as a research problem, so the physician must develop the investigative viewpoint in the study of his patient, utilizing to that end as many and as varied avenues of approach and study as the diagnostic and therapeutic problem in that case may necessitate.

Having thus by these broad generalizations prepared the way for a more particular discussion, we may now discuss as briefly as may be the utilization and significance of various blood examinations in the study of disease. In so doing, we shall not select the unusual nor the ultrascientific, but rather the more common and homely blood studies which, perhaps because they are familiar, are not always appreciated or utilized to the fullest extent.

THE BLOOD COUNT

The blood count coupled with the cytologic study of the stained smear is obviously of paramount importance in the study of primary disorders of the blood-forming organs. But primary disorders of the hematopoietic system are rare, while disturbances of the blood as a concomitant or aftermath of disease in general are so common that it can be said with confidence that there is hardly any disease in which a systematic hematologic study will not prove of material aid and value.

As everyone knows, an accurate diagnosis of anemia is impossible without a blood count, for symptoms cannot be relied upon. Pallor of the skin is entirely unreliable because it depends, not only upon the amount of hemoglobin per unit volume of blood, but also upon such varied factors as the skin thickness, the amount of skin pigment, the presence or absence of edema, the state of contraction or relaxation of the capillaries, and the degree to which the individual is exposed to or protected from ultraviolet radiation.

In like fashion other symptoms associated with anemia, such as weakness, shortness of breath and edema, may be present in many other conditions and absent in the presence of anemia.

To recognize an anemia, therefore, and especially to recognize its type and, by inferential deduction the underlying responsible mechanism—an understanding of which is essential to intelligent treatment—recognition must be had to examination of the blood.

Reliance upon hemoglobin determinations alone, as is sometimes done is not sufficient and may, indeed, prove misleading, a simultaneous study of the red cells is essential.

It is now clear that the production of anemia depends upon two fundamental factors—an increased rate of loss or destruction of red cells or a decreased rate of formation either of which may be brought about by a variety of causes.

From the standpoint of efficient treatment the necessity of determining not only the presence of an anemia but its type is apparent. Fortunately, this can be determined by a careful study of the characteristics of the red blood cells, so that, from this angle, the most important feature of the blood count is not the

amount of hemoglobin and the number of erythrocytes, but that portion of the report describing what is seen in the stained smear.

We now divide anemias into three main groups: normocytic, macrocytic, and microcytic, in accordance with the size of the erythrocytes: normal, larger, or smaller than normal.

This is of therapeutic as well as academic importance. For all macrocytic anemias depend upon a deficient supply to the bone marrow of the antipernicious anemia principle, and hence respond to the administration of this principle. Microcytic anemias, in which the cells are hypochromic as well as smaller than normal, are largely dependent upon an insufficient supply of the substances essential to hemoglobin formation, of which iron is the most important. Hypochromic microcytic anemias may thus be expected to respond to iron therapy. Macrocytic anemias, on the other hand, can be produced in a variety of ways, and their etiology must be determined before intelligent therapy can be planned.

Important and valuable as are these simple procedures, there are others of ancillary and sometimes great importance in the study of a particular problem. Among these are the various indices of color, volume, and saturation.

The color index expresses the mean hemoglobin content of a single cell as compared with the content of a normal cell. The volume index expresses the ratio between the average size of the red cells in the blood examined and the average size of the red cell in a normal blood. The saturation index expresses the relation of the mean corpuscular hemoglobin concentration to normal and hence indicates the relation of the hemoglobin concentration to cell size.

Under normal conditions all three indices range between 0.8 to 1.2 and hence will be found within this range in normocytic anemias which are not characterized by general changes in the size of the red cells.

In the macrocytic anemias, where the erythrocytes are large, the volume and color indices will naturally exceed normal, while the saturation index remains within the normal range or shows but a slight increase.

In the hypochromic microcytic anemias all the indices, as can be foretold by the type of anemia, are below the normal range.

If blood studies are thus seen to be important and essential for the accurate diagnosis of anemia, and of equal importance at times in indicating the type of therapy most likely to be efficient, they are also of great value as a measure of control of the results of therapy.

In the icterus index, assuming absence of hepatic disease or biliary obstruction, we have a gauge of the degree of erythrocyte destruction; while the reticulocyte count furnishes an equally accurate index of the degree of red cell regeneration.

In the absence of hepatic or biliary disease an icterus index above 6 indicates increased red cell destruction, the index increasing as the erythrocyte destruction increases.

A reticulocyte count of less than 0.5 per cent is an index of abnormally slow regeneration; a count of 4 per cent or over in individuals over 4 years old indicates an increased rate of erythrogenesis, and this procedure may thus be utilized as a measure of therapeutic response. However, as the investigations

of various workers have clearly shown, the degree of reticulocyte response to therapy is governed not only by the activity of the therapeutic agent and the adequacy of the dose, but also by the initial level of the red cell count and can only be used as a therapeutic gauge when the initial red cell count is 3 million or less. Above this initial level reticulocyte counts are irregular and unconstant.

The reticulocyte count is also of some value in differential diagnosis, being increased in sickle cell anemia, acute febrile hemolytic anemia (Lederer's anemia), familial hemolytic icterus, myelogenous anemia, lead poisoning, malaria, and any anemia characterized by an increased rate of blood destruction.

A marked, but transient increase in the reticulocyte count follows acute hemorrhage and, occurring in pernicious anemia, forecasts the approach of a spontaneous remission.

Even when a reticulocyte count is not done a definite, but by no means as accurate, idea of the presence or absence of regenerative activity and a rough approximation of its degree may be had from a study of the stained smear with particular reference to the presence, absence, or incidence of polychromatophils (of which basophilic stippling is one manifestation) and nucleated red cells, all of which represent phases of erythrocytic formation.

It is, of course, unnecessary to dwell upon the vital importance of the blood count—in which is included the study of the stained smear—in the diagnosis and study of diseases of the blood itself, such as polycythemia vera and the leucemias.

Blood Count in Concealed Hemorrhage—The blood count is not infrequently used as a means for the detection and measurement of concealed hemorrhage, but not always used to the best advantage. For example, a patient may present signs and symptoms suggesting but not clearly, the possibility of some abdominal catastrophe accompanied by hemorrhage, such as a ruptured ectopic or a laceration of an organ.

Not infrequently, information for or against such a diagnostic possibility is sought by means of repeated serial blood counts or by repeated determinations of hemoglobin and red cell counts. Such use does not always take into account the sequence of events following hemorrhage.

In the case of relatively small hemorrhages the effect upon the hemoglobin and red cell count may be so minor as to be unimportant and inconclusive until, as a result of long continued demand, evidence appears of fatigue on the part of the hematopoietic system, and this, of course, is of little use where the emergency is acute.

In the case of relatively large hemorrhages, no change will be seen in hemoglobin determinations or red cell counts until some hours—and sometimes quite a few hours—later when both will drop as a result of dilution of the blood by the fluids withdrawn from the tissues to compensate for the fluid lost in the hemorrhage. Here again, the effect may appear too late to be of aid in an acute emergency, such as those just cited.

In such case, the important information of clinical use and value is to be obtained, not from a study of the hemoglobin and red cells, but from the total and differential counts. For hemorrhage into a closed cavity lined with an absorbing membrane such as the peritoneum produces an early and marked

polymorphonuclear leucocytosis, and this finding, showing a progressive leucocytosis, may be used with confidence as a sign of concealed hemorrhage in such localities.

Blood Count in Infections.—While many infectious diseases are capable of producing more or less anemia, the most characteristic, most definite, and most diagnostically valuable changes are seen in the alterations produced in the number and characteristics of the leucocytes and this discussion will therefore be confined to these.

It is now many years since Sondern first enunciated the now commonly accepted rules that the severity of an acute infectious process was in large measure suggested by the percentage of polymorphonuclear leucocytes in the differential count, and the reacting ability of the patient in large measure suggested by the total leucocyte count.

Oddly enough, these two axioms still suffice for some to the utter neglect of the many and important refinements now associated with the study of the leucocytes in the presence of infection.

While the dicta just mentioned are in large measure true, they cannot be blindly relied upon for the following rather obvious reasons:

In the first place leucocytosis and leucopenia are primarily manifestations of reaction to stimuli, just as, indeed, are all the varied manifestations of disease which we call symptoms.

There are, however, several variable factors involved in the production of these phenomena. One is the nature and intensity of the stimulus; another the ability of the patient to respond to it. Obviously, therefore, alterations in the total and differential leucocyte count will depend, not only on the presence of infection but also, and to no small extent, upon the ability of the patient to respond to the stimuli exerted by the phenomena attendant upon the pathologic processes resulting from the disease.

A diffuse and spreading infectious process may be expected to be accompanied by leucocytosis, both total and polymorphonuclear, unless the toxic absorption is so massive or the infection so virulent that the patient is overwhelmed. In such a case the total leucocyte count may approximate normal, or there may even be a leucopenia.

On the other hand, a severe and virulent infectious process may be so located and so well walled off that absorption from it, and hence stimulation, may be reduced to a minimum, and the leucocytic response correspondingly less marked.

Thus it is that, as is always the case in any method of examination of the patient, among which are laboratory procedures, the import and value of the procedure depends, not upon its results, but upon their interpretation.

It would not be safe, for example, to base a diagnosis of acute lymphatic leucemia upon a leucocytosis of 100,000 and a lymphocytosis of 98 per cent in a child, because such a picture may be encountered as an evidence of reaction to pertussis vaccine. Similarly, a diagnosis of myelogenous leucemia cannot be safely based upon a high total count and the presence of myeloblasts, for such findings may occur as what has been termed the "leucemoid reaction" to infection.

Failure to obtain from the leucocyte counts the utmost in clinically useful information arises from various causes, among which may be mentioned, the paying of too much attention to the cells which are increased and not enough to those which are decreased

An old but pertinent observation often forgotten nowadays, relates to the effect of infections upon the eosinophiles. A decrease in the eosinophile count in conjunction with an increased polymorphonuclear percentage was recognized as indicative of a pyogenic process as long ago as 1902. This was the so called "septic factor" which has definite value, so much so that a return of the eosinophile has a definitely favorable import.

The next step in the study of the leucocytic response to infection was the endeavor of various investigators to reduce the leucocytic reactions to formulas, resulting in various graphic reports such as the Gibson chart, Walker's index of body resistance, the leucocyte index of Kiebs and so on. All of these had more or less value when properly computed and interpreted, but have now largely been discarded in favor of methods based upon the pioneer work of Arneth and its modifications by Schilling and others so that the leucocytic hematology of infections is now largely dominated by the hemogram depicting the presence or absence of "shifts."

At first glance, such formulas appear somewhat complex, particularly as presented by some workers. In the ultimate analysis however, they are based upon relatively simple principles.

It is apparent that the mobilization of leucocytes in response to infectious stimuli must necessitate the entrance of these cells from the various depots in which they are formed and stored. Where the stimulus is slight, the mobilization of mature cells may suffice. When this does not suffice, immature neutrophils appear in the peripheral blood in proportion to the intensity and urgency of the demand.

Arneth was the first to call attention to the variations in the shape and character of the polymorphonuclear nucleus, and to associate these changes with the age of the cell in question, these investigations being later extended and revised by Schilling and others. All of these workers, in tabulating the various stages of nuclear morphology, naturally listed the youngest types first, followed by the other types in their supposed order of age and maturity.

When percentage estimations were made of these various immature forms it was found that the proportion of younger forms was increased in proportion to the intensity of the reaction, and that hence the highest figures were seen under the youngest cells at the left of the form, and this finding was somewhat slangily expressed as "a shift to the left" to indicate a preponderance of immature leucocytes. From these relatively simple beginnings there has arisen an extensive hematologic structure with many complicated ramifications.

While there is no question as to the value of studies of leucocytic nuclear morphology in disease in general and in infectious diseases in particular, there is some room for question as to whether or not the value of the procedure is enhanced by the complicated and sometimes even cumbersome nomenclature which has grown up about it.

It is for this reason that many attempts have been made to modify the methods of reporting such studies so that they may be made more simple and rapid in performance and especially more simply reported.

One of the simpler and yet very useful methods of determining the presence or absence of a shift, as well as its degree, is by dividing the total number of mature cells by the total number of immature cells found, the resulting figure being called the "nuclear index" (Boerner).

In the adult the normal index is 15 or over, an index of 10 to 15 denoting a very slight shift to the left; 5 to 10, a moderate shift to the left; and an index under 5, a marked shift.

The impossibility of elaborating leucocytic formulas applicable under all situations and of invariable significance is, of course, apparent. We must be prepared, then, for qualifications in the interpretation of variations in nuclear morphologic determinations, no matter how they may have been made.

For example, because of the extreme mobility of the bone marrow in infants and children, and the consequent even marked response to minor stimuli, such counts cannot be interpreted as in the adult, although an approximate basis for interpretation may be had by subtracting about 5 per cent from the immature neutrophilic percentage count in children 4 to 12 years of age, and about 10 per cent in infants under 4 years of age.

Likewise, these methods are inapplicable to noninfectious diseases, leucemias, pernicious anemia, and other myelophthisic anemias in which the presence of immature neutrophils has no definite relation to prognosis. Nor can they be used in malaria because of the common occurrence of large numbers of young (staff) cells.

The interpretation of leucocytic variations may be summarized somewhat after this fashion.

1. A slight nuclear shift associated with a moderate neutrophilic leucocytosis and a persistence of eosinophiles is, when this picture is sustained, of favorable import.

2. A moderate nuclear shift in the presence of a slight leucocytosis, coupled with a decrease of eosinophiles and lymphocytes, warrants the diagnosis of infection, but is without prognostic significance.

3. A high leucocytosis associated with a marked shift, an absence of eosinophiles, and a decrease in lymphocytes, is of ominous import.

4. When, in the presence of lymphopenia, an absence of eosinophiles, and an increasing shift to the left, there is also a progressive fall in the total leucocyte count, the picture warrants a fatal prognosis with a considerable degree of certainty.

5. An eosinophilic leucopenia, when coupled with a rising total count, signifies a pyogenic infection; in the presence of a falling total count it suggests lack of resistance.

6. Lymphopenia coupled with a rising leucocytosis suggests a progressive inflammatory process; with a falling total count the prognosis is ominous.

7 Lymphocytosis associated with a return of the total count and nuclear index to normal is the usual accompaniment of convalescence

Generalizations applicable particularly to variations in the nuclear morphology may be thus briefly summarized

1 The peak of the immature cells is coincident with the height of the infection As the disease process lessens and convalescence sets in, red forms return to the normal level of 3 to 5 per cent Then persistence at a high level suggests some complication

2 In pneumonia a staff count of nearly 50 per cent of the total frequently accompanies the crisis When the number of immature forms approaches 50 per cent in early pneumonia, the prognosis is ominous

It will be noted that in all discussions of this matter of nuclear variation in the study of disease the bulk of the emphasis is laid upon the shift to the left It should not be overlooked that the reverse of this, or a "shift to the right"—in other words, the return of mature leucocytes to the blood picture—is of some value as predieating the return to normal

From even this cursory survey it is seen that while all these various refinements in the study of the leucocytes have greatly increased the prognostic significance of the leucocyte count, the procedure is still mainly a measure of activity and response to stimuli, with marked enhancement in differential diagnostic and prognostic significance

So much emphasis has been laid upon the study of the nucleus that the value and significance of the study of the leucocyte as a whole has been somewhat overshadowed, except to the hematologist

Changes in the cytoplasm and granules of the granulocytic leucocytes have been so definitely found, so clearly described, and their significance so definitely indicated that the hematologist now recognizes the "toxic neutrophile" and utilizes it as a valuable prognostic indicator

The changes denoting the toxic neutrophile are a tendency of the cytoplasm to take a bluer stain than normally, the presence of vacuoles in the cytoplasm, and variations in the size, number, and staining reaction of the granules, the toxic granule being larger, bluer, and not so numerous as in the normal cell

Any one of these changes may be present alone or in combination, and, of course, may vary in degree When present it is customary to grade their degree, as compared to the deviation from normal in the average cell of the patient's blood, by the arbitrary use of one to four plus

As a rule, the presence of toxic neutrophiles is associated with a marked increase in the platelet count and their evaluation has been found to be of great prognostic value When two of the changes indicative of the toxic neutrophile are present in three to four plus degree, a fatal outcome is almost inevitable

The absence of toxic neutrophiles has not the reverse significance, however, as occasionally patients fail to develop such changes even up to the time of death

Also, it is to be borne in mind that caution must be used in evaluating the significance of toxic changes in peritonitis and pneumonia, as variations of from three- to four plus in any two of these changes indicating toxicity occur quite constantly in these two conditions with subsequent recovery

Some workers calculate the percentage of neutrophils showing toxic changes and report this as the degenerative index. It is practically as easy, however, and rather definitely more valuable, to estimate the degree of all the toxic changes noted and to base prognostic inferences upon this.

BLOOD SEDIMENTATION

Among the relatively recently developed procedures which has an accepted place in the field of laboratory studies is the sedimentation test.

Time does not permit discussion of the various theories advanced to explain its mechanism nor to mention the various technical factors which may produce anomalous and even fallacious results. It must be emphasized, however, that the blood sedimentation rate is a nonspecific phenomenon, entirely without specific diagnostic significance, and constituting but one of the varied manifestations of the reaction to pathologic stimuli. Its lack of specificity is apparent from the fact that the sedimentation rate is increased in greater or lesser degree in such varied conditions as pregnancy, neoplasms, and infections.

The sedimentation test derives its chief clinical value from the fact that it is a reliable and delicate indicator of activity, so that an increased sedimentation rate signifies an active process, whatever it may be, and a return toward the normal rate of sedimentation indicates a decreasing activity in the pathologic process. There are no specific sedimentation curves characteristic of any specific condition.

From the clinical standpoint the sedimentation curve has a very definite, though somewhat restricted, field of usefulness:

First, in gynecology as a means of determining when a tubal inflammatory process has become quiescent and thus most suitable for an elective operation. It has been suggested that there is a specific difference between the sedimentation rate in salpingitis and that in appendicitis, enabling a differential diagnosis between the two conditions, in that an increased rate appears earlier in salpingitis than in appendicitis. This observation awaits corroboration.

Second, in the management of ambulant tuberculosis where an increasing sedimentation rate is often the first indication of a reactivation of the tuberculous process.

Third, as a means of aiding in the differentiation of inflammatory from noninflammatory processes in, for example, the chest, in arthritides, and in the genitourinary system.

Fourth, as suggesting pregnancy in the absence of neoplasms and infectious processes.

Fifth, in distinguishing organic from functional disease in the gastrointestinal tract. It is a wise axiom never to diagnose a functional neurosis in the presence of an abnormal sedimentation rate.

Finally, there is evidence to suggest that, for reasons unknown, the sedimentation rate is either normal or decreased in pertussis, even though this is an acute febrile disease. Hence, in the presence of cough associated with a leucocytosis and marked lymphocytosis a coincident normal or decreased sedimentation rate suggests pertussis as a diagnostic possibility.

The sedimentation test has been applied in the presence of almost all known diseases, and in the early days of its use many statements were made which the test of time has failed to substantiate. While its mechanism is still largely unknown, it is practically certain that it is definitely related to tissue destruction, which, of course, explains its innumerable variations in innumerable conditions as a measure of activity.

It should be emphasized that inferential deductions drawn from the sedimentation rate are best and most safely and accurately based, not upon a single determination but upon the trend of a series of determinations.

AGGLUTINATION REACTIONS

Following the World War the rapid increase in the number of individuals vaccinated against infections of the typhoid group complicated somewhat the clinical interpretation of agglutination reactions. The problem appeared to become still further complicated by the more recent studies indicating that, in accordance with certain specific differences in typhoid strains, there were differences in the type of agglutinins produced and, consequently, in the character of the resulting agglutination reactions.

The discovery as long ago as 1917 that colonies of a pure culture of bacteria might vary in their appearance, some being smooth and shiny and others rough and more or less opaque, was soon followed by appreciation that these cultural differences were accompanied by certain other differences, among which were perceptible differences in the type of agglutinins produced by these two bacterial types.

When a suspension of a smooth or S-type organism is brought in contact with a specific agglutinating serum, the resulting agglutination takes the form of large flocculent clumps. When a suspension of a rough or R-type strain is agglutinated, the agglutination takes the form of small compact granules falling to the bottom of the tube.

S-agglutination occurs rapidly, settles slowly, and is flocculent in character. R-agglutination forms slowly, settles rapidly, and is granular in appearance.

Without entering into the many minutia of these studies it suffices to say that it has now been conclusively demonstrated that the granular type of agglutinin is thermostable and of somatic origin, while the flocculent type of agglutinin is thermolabile and flagellar in origin. At first glance all this might seem to be of little or no clinical importance, but its practical significance becomes apparent from these facts:

1. Formolized suspensions for agglutination reactions respond mainly to the flagellar type of agglutinins, while alcohol-killed suspensions respond mainly to the somatic type of agglutinins.

2. While in the majority of instances both flagellate and somatic agglutinins are produced in typhoid fever, in some only the somatic type of agglutinin is produced, in which case there would be little if any response with a formolized agglutination suspension.

It thus appears that the thorough study of diseases of the typhoid group by means of the agglutination reaction may in the future necessitate the routine

use of both alcohol-killed and formalin-killed suspensions in order that both types of agglutination may be detected and quantitatively measured.

This becomes of clinical interest because of the fact that, in a general way, the agglutinins formed as a result of typhoid vaccination are largely of flagellar origin, while those resulting from an actual infection are largely of somatic origin.

Agglutination of the alcohol-killed suspension, therefore, usually suggests typhoid fever or infection with a closely allied species, while agglutination with a formalinized suspension, and hence of flagellar type, suggests one of three possibilities: the patient has typhoid fever, he has had the disease in the past, or he has been vaccinated.

The question is sometimes asked, "What strength of agglutination titer is diagnostic or suggestive of infection?"

The answer is clear and definite: No arbitrary titer can be selected which is positive in the diagnostic sense or below which it can be regarded as diagnostically negative. An agglutination reaction is simply one item of evidence to be considered in relation to all the other evidence available.

Certain general principles may be laid down, however, to govern the clinical utilization of this procedure:

1. Given a patient presenting symptoms suggestive of enteric fever—which, it must be remembered, includes infection with any of the organisms in the *Salmonella* group (of which there are about twelve species capable of causing continued fever)—a negative reaction to the suspensions used may signify that: (a) the patient does not have enteric fever; (b) that the specimen was taken before agglutinins appeared; (c) that the disease is of unusual type and caused by an organism not represented in the suspensions used. The first two alternatives are differentiated by a repetition of the test; the last by a resort to attempts to isolate the organisms by culture.

2. A negative reaction in the second or third week of the disease is of definite negative diagnostic value and repeated negative reactions render the clinical diagnosis unlikely.

3. A positive reaction must be considered in relation to (a) the distribution of agglutinins in the normal population, (b) the stage of the disease at which the test is made, and (c) the history as regards vaccination.

4. As is usually the case with many laboratory procedures, the trend of a series is of greater value and significance than a single determination.

5. Quantitative reactions are far more clinically valuable than tests with one or two arbitrarily selected serum dilutions.

6. It is a wise precaution to include a suspension of *B. abortus* in the routine Widal set-up and to use a *B. tularensis* suspension in the study of any obscure continued fever.

BLOOD CHEMISTRY

Discussion of this phase of laboratory investigation must needs be brief and may be prefaced by the statement that the average hospital laboratory expends much time, labor, and material in the conduct of chemical determinations of little clinical value and which furnish little or no useful information.

It may also be emphasized that requests for "blood chemistry" or "routine blood chemistry" are meaningless. Every requisition for blood chemistry determinations should be specific and should have in view the acquisition of specific information relating to various phases of functional efficiency applicable to the corroboration or negation of a diagnostic possibility. This, indeed, applies to all laboratory requisitions.

The following generalizations are applicable:

1 As the urea nitrogen in health and disease, is always approximately 50 per cent of the nonprotein nitrogen, it is unnecessary to order both. Determination of the urea nitrogen is preferable as simpler and more rapidly performed.

2 Creatinin need not be determined unless the blood urea is over 30 mg per cent.

3 There is only one real indication for the determination of uric acid: gout or the suspicion of gout.

4 Blood calcium determinations are routinely indicated only in the presence of tetany, sometimes in the presence of spasmodophilia associated with rickets, inasmuch as the blood calcium in rickets as a rule, is normal, rarely in fractures when nonunion is thought to be associated with disturbances of calcium metabolism, and infrequently as a method of regulating parathyroid hormone therapy.

5 Phosphorus determinations are useful only in the study of tetany and rickets.

6 Chloride determinations find their greatest value in the study of the toxemias associated with disturbances of gastrointestinal motility. They are of little value or significance in nephritis.

7 Indications for determinations of carbon dioxide combining power are (a) diabetic patients with acetone in the urine, (b) uremia with nitrogen retention and dyspnea, (c) toxic patients receiving alkali treatment, (d) tetany of all types, and (e) disturbances of gastrointestinal motility with toxemia.

Blood Culture—It is not only wise but profitable to resort to blood cultures in (a) any disease known to be characterized by bacteremia, (b) in any condition where the clinical picture and symptomatology suggest bacteremia as a diagnostic possibility, (c) in any sustained febrile reaction the etiology of which is obscure, and (d) in certain acute infections, such as pneumonia or meningococcal meningitis for example in which the demonstration of bacteremia is of pronounced prognostic significance.

The success or failure of bacteriologic examinations of the blood is determined by a variety of factors, some of which arise from the inherent characteristics of the disease in question, others dependent upon the particular method or methods employed, still others related to the characteristics of the bacteria in question, and, finally, those arising from the circumstances under which resort is had to this procedure.

In those diseases characterized by bacteremia, of which the typhoid group is the classic example, it is well recognized that the earlier in the disease the culture is taken the greater will be the incidence of positive results. The reason for this lies in the fact that in every bacteremia the entrance of the bacteria is

from some initial focus, that this entrance may be, and probably is, more or less intermittent, and that the bacteria are more or less constantly being removed from the blood stream by the varied mechanisms of defense.

In bacteremia the bacteria do not make continuous circuits of the blood stream reproducing as they go. On the contrary, not only do antibacterial substances arise and travel with them, but they are subject to mechanical removal by filtration, by lymph nodes, by deposition in various tissues and organs which may later function as foci of secondary invasions, and by phagocytosis.

As a result of these interacting forces it is probable that there is a rise and fall in the bacterial content of the blood dependent upon repeated influx from the primary as well as the secondary foci and influenced by repeated attempts of the defense mechanisms to cleanse the blood stream.

It is apparent, therefore, that the time at which the culture is taken is of definite importance and definitely influences the result. The most propitious time is hence as soon as possible after the clinical signs suggestive of bacterial invasion of the blood appear, namely, chill, rigor, and rise of temperature.

While the clinical pathologist usually inoculates a blood culture into a variety of media calculated to secure growth even when the organism in question is selective in its cultural requirements, it is obvious that knowledge of the presenting possibilities enables him to vary his methods accordingly and so enhance the chances of success. It is not only advisable, therefore, but of definite value that requests for blood cultures be accompanied by the tentative clinical diagnosis or some statement concerning the clinical features of the case.

Under certain conditions, such as in puerperal septicemia, for example, the invading organisms not infrequently, if not strictly anaerobic, grow best under conditions of partial tension. All blood cultures, therefore, should include anaerobic and partial tension methods.

Blood cultures should be so devised as to furnish information, not only of the presence of bacteria in the blood, but some measure as to the degree of bacterial invasion. In a word, they should be quantitative, and this can easily be done so that reports can be made in terms of the number of colonies per cubic centimeter of blood, thus furnishing the clinician with an estimate of the gravity of the condition of value from the prognostic as well as the therapeutic standpoint. Such a procedure in later cultures also serves as a measure of the response to treatment.

As a rule, after a preliminary report after twenty-four hours, blood cultures are not finally reported upon until they have been held for seven days. Sometimes, however, especially in subacute bacterial endocarditis, cultures must be held for three weeks or more before the final report can be made. In this condition it is hence imperative that the clinical pathologist be aware of this diagnostic possibility in order that he may not discard his cultures too soon.

The rapidity with which growth occurs in blood cultures is determined both by the ease with which the bacteria in question grow on artificial media, and also by the number present in the blood at the time the examination is made. Growth, even of organisms relatively easily propagated, may not be perceptible, therefore, for some little time.

The significance of negative blood cultures is governed by various factors among which may be noted

1 The character of the infection In subacute bacterial endocarditis, for example, where the blood stream invasion is not only intermittent but often of minor degree, several attempts may be necessary before growth is obtained

2 The time at which the culture is taken The further from the time of the invasion, the lower will be the incidence of positive cultures

3 The duration of incubation as well as the media and methods used are of definite influence and emphasize again the value of informing the clinical pathologist of the possible diagnosis

The demonstration in a blood culture of organisms not commonly encountered in the skin is positive evidence of a bacteremia When staphylococci are found, however, and especially when they are present in small numbers or only in one or two media, the culture should be repeated in order to rule out skin contamination

Staphylococci in large numbers in all media are usually evidence of staphylococemia, although it is wise to check this finding by a repetition of the culture

Flocculation Tests—The present inauguration of a campaign against syphilis has focused attention anew upon the flocculation reactions which have been developed in recent years as methods for the serologic detection and study of syphilis That they are reliable delicate and useful adjuncts in the serologic study of syphilis is generally recognized What does not seem to be equally as generally emphasized however is the fact that as is true of any reaction which depends upon an interaction between the patient and his disease, these procedures have their limitations

Much has been made of the fact that flocculation tests may be rapidly performed and especial emphasis has been laid upon their simplicity of technique Indeed, so much has been said of these qualifications that, by inference or even direct statement, the impression has been spread that anyone from the janitor to the serologist may satisfactorily perform such tests, and they are being widely hailed as "office tests" that anyone can do In fact, a prominent biologic supply house is now advertising a 'new flocculation test which can be completed in ten minutes, which—according to the advertisement—requires little, if any skill and which can be performed for an average cost of two cents' And another general supply mail order firm advertises the equipment for one of the older methods for \$9.75

It seems advisable and timely to recall some pertinent facts

In the first place, there is evident danger that the public, as well as the physician, may be led to believe that the Wassermann test, or its equivalent, is all that is necessary to diagnose or rule out syphilis, from which it follows—if some recent trends in the literature and especially in the advertising "literature" directed to the physician are to be believed—that anyone on the sole basis of a ten minute office procedure can label a patient with the stigma of syphilis and condemn him to from two to five years of treatment, or, on the contrary, release a potential focus of infection on the same grounds

It is not unprofitable, in this connection, to recall, from an address by Dr. James Gregory, Professor of Medicine in the University of Edinburgh from 1790 to 1821, the following:

"I do not know, nor can I conceive, any human contrivance that can more effectually and irresistibly oblige the physician to study carefully the case of his patient; to attend to every symptom and change of symptom; to exert himself to the utmost for his patient's relief, and at the same time be as careful as possible in the remedies he employs; than to find himself under the necessity of giving a minute account of everything he has done in a very public manner and before a number of competent judges."

Under such circumstances it is not unreasonable to suppose that there would be some embarrassment attached to a diagnosis of syphilis largely based upon a ten-minute-test and ten cents worth of reagents!

Now, while it cannot be disputed that serologic procedures are of paramount importance in the study of this disease, it must be remembered that careful histories, meticulous examination, and their careful correlation are equally important and equally essential. The old adage to "be quick to suspect syphilis but slow to diagnose it" is still in good standing.

In the second place, while it is true that the flocculation tests are simpler in technique than the complement fixation tests, behind these simple manipulations are the same basic principles underlying the complement fixation tests, principles none of which are simple, all of which are intricate and complicated, and some of which are yet incompletely understood.

It is important that workers in the field of serology understand them, as far as they can be understood, because of the fact that *all* laboratory procedures may go awry at times, and it takes an experienced worker with a thorough fundamental training both to appreciate when trouble begins and especially to find and eliminate the cause.

In the third place, the laboratory procedure itself is never as important as its interpretation, especially in the borderline case, in which neither the reaction obtained nor the clinical significance to be attributed to it are clear-cut.

It seems questionable, to say the least, whether perusal of the advertising "literature" accompanying the outfits now offered for sale for the performance of these "office tests" can justly be regarded as conveying sufficient information to make of the reader an efficient serologist or a competent syphilographer!

One may also be permitted to doubt whether or not rapidity and cheapness would be the paramount factor in determining the diagnosis of syphilis on one's own blood, even though hailed as sufficient for the patient.

It must be remembered that while the diagnosis of syphilis, with all that it implies, is very easily made, it is far from easily removed. The point need not be labored.

Even so rambling and discursive a discussion as this will serve to indicate, in some measure, the diversity of the present avenues of approach to the study

of disease. It may also serve to suggest something of the many factors upon which the clinical utilization and interpretation of laboratory findings depend.

It may, perhaps, again be emphasized that laboratory procedures, although commonly—and somewhat unfortunately—spoken of as ‘tests’ are, of course, not tests for the presence or absence of disease but specialized phases of the examination of the patient. While this need not be emphasized to the physician, it may with profit be called to the attention of the patient. For, thanks to the advertising methods of the lay owned and lay operated commercial laboratories and to the implications surrounding the drugstore laboratory the public has become more or less ‘laboratory conscious’.

By this it is not meant that the public has become trained to expect of the physician the proper utilization of all that modern medicine has to give to the study of disease, but rather that there has developed a more or less widespread tendency to regard the laboratory and its procedures as a sort of mystic divining rod for the detection of disease.

Every clinical pathologist encounters individuals who come to the laboratory for a ‘urine test’ or a ‘blood test’ to find out whether or not they are healthy, and has been obliged to expend valuable time in explaining, not always successfully, the futility of such procedures unless they are deemed indicated by the physician and subject to his interpretation. The laity perhaps are not altogether to blame in this matter for medicine to no small extent is still some what shrouded in the mantle of mystery which it inherits from the past.

The necessity in these modern days for extensive use of laboratory methods has, by what Webster has called ‘the fearful concatenation of circumstance,’ resulted in a mushroom growth of laboratories not infrequently owned conducted, and manned by lay personnel trained solely in the purely manipulative features of laboratory procedures. Indeed all too often the laboratories clamoring for the physicians’ business are aggressive in proclaiming that ‘the test is the thing’ and seek by an obtrusive display of glittering apparatus and the clamorous use of technical patter to varnish nonsense with the charm of sound.”

It is not altogether surprising that the average layman, to whom the clerk in the drugstore, the good fellow who handles his favorite brand of cigars, and the physician alike are known as “Doc,” should regard the technician as a medical oracle.

The physician, of course, knows that technical skill and experience no matter how extensive, do not make a clinical pathologist. On the contrary the value of the clinical pathologist in the study of disease is entirely dependent upon and entirely in proportion to his clinical as well as laboratory ability to aid, when called upon not only in the selection of suitable laboratory studies applicable to a particular problem but also, at times, to aid in their clinical interpretation and utilization.

For so complex may clinical problems become that one is reminded some times of the words of General Wolfe in one of his dispatches to Lord Pitt: “There is such a choice of difficulties that I find myself at a loss how to determine.”

STUDIES ON ANAPHYLAXIS WITH INSULIN*

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INTRODUCTION

INVESTIGATION of the physiologic effects of insulin has so completely engaged the research interest of workers in this field that little attention has been directed to its immunologic properties. These properties have been brought into increasing prominence by the reports of unusual reactions such as are seen in insulin-resistant¹ cases or in the insulin sensitive individuals who present urticaria, edemas, asthma, or other untoward manifestations following its injection. Minor allergic responses, such as "dwarf" urticaria are met with not infrequently, but despite the wide use of insulin, grosser sensitization, or anaphylaxis, is still uncommon enough to occasion several accounts of this condition annually.²⁻⁶ Indeed, those who follow any large group of allergic individuals are uniformly impressed by the paucity of diabetics in their clinic population.⁷⁻⁹ This observation has been the source of various theories regarding the diabetic constitution, and one wonders whether the latter is antithetic to the allergic constitution, or whether, genetically, the simultaneous appearance of the two traits constitutes a "lethal factor." One would not have the temerity to defend completely this latter hypothesis, especially in view of the actual, albeit rare occurrence of the two conditions in the same individual. Speculation regarding the mechanism of insulin resistance has also stimulated interest in its immune processes. Is insulin fixed in situ in such cases and slowly inactivated, or does the union of antigen and antibody immediately inactivate it? Does its combination with the fluids or tissues of the host under particular but as yet unknown conditions reduce its hypoglycemogenic effect? We do not presume in this communication to answer all these questions, many of which are in the present state of our knowledge necessarily rhetorical. However, some inquiry into the immunologic properties of insulin seemed indicated, and we report the results of our investigation on this point. Data from work pursuing the subject further will be recounted in a later communication. The present series consisted of several groups of animals of which one representative study is set forth below.

MATERIALS AND METHODS

The guinea pigs were males, weighing between 250 and 300 gm. Injections with Luer syringes were made intracutaneously with some possible subcutaneous leakage. Normal horse serum was obtained from Eli Lilly Company, whose commercial insulin U 40 was also used

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in the experiment. A highly purified commercial preparation of insulin U 233, derived from beef pancreas, and containing 1580 gm nitrogen per cubic centimeter was supplied by the Research Division of E. R. Squibb and Sons. We are indebted to Dr. E. M. K. Geiling and particularly to Dr. H. Jensen of the Department of Pharmacology, Johns Hopkins University Medical School, for a generous quantity of powdered crystalline insulin used in these studies. Frederick Stearns and Company furnished us with their crystalline insulin U 40 (nitrogen content 14 per cent dry crystal—zinc content 1 m. per 500 units). The omission of any reference in the tables to differentiate either type is intentional for purposes of simplification. The results with the two crystalline products were identical in every respect.

EXPERIMENTAL

We were first interested in the possibility of sensitizing guinea pigs to insulin. The method employed by one of us in pollen sensitization was first used. This consisted in the use of a mixture of equal parts of normal horse serum and, in this instance, insulin U 40. The mixture was allowed to stand at room temperature for twenty-four hours and then kept at icebox temperature for further use. At four-day intervals guinea pigs were injected subcutaneously with 3 c.c. of this horse serum-insulin mixture and after a total elapsed time of twenty-eight to thirty days were ready for the test of anaphylaxis. Other guinea pigs were similarly injected with 0.2 c.c. commercial insulin alone (same lot). Several animals were lost through hypoglycemic shock or intercurrent infections and necessary modifications in dosage may be observed in the tables. It was soon apparent that any sensitization induced to insulin was in no consistent manner dependent upon the horse serum component of the "combined" antigen, and this phase of the investigation was not extended. However, the initial anaphylaxis was greater in this group and is commented upon later. Other guinea pigs were divided into groups dependent upon the type of insulin used for sensitization and for shock, and upon the method of sensitization. They were shocked at three and six weeks in intervals after the last sensitizing dose by intravenous injection into the exposed jugular vein. The amounts used were 0.2 to 0.4 c.c. of insulin containing from 4 to 19 units as indicated. Reactions were carefully observed and were classified as described in the legend (Table I). Typical anaphylactic responses were observed within five minutes after injection. These consisted, in increasing order of severity of nervousness, rapid breathing, cratching of nose, dyspnea, cyanosis, wheezing cough, loss of posture, pre-tetanic convulsions and death. Any reactions occurring after forty-five minutes were discounted as being possibly hypoglycemic and were considered as insulin shock. This latter type of reaction was slower and more gradual in its appearance. The animal first became listless, weak, and apathetic, and, if not given glucose, eventually exhibited convulsive movements. To combat the latter, intraperitoneal injections of 50 per cent glucose in physiological saline were employed.

It can be seen from Tables I and II that animals receiving insulin alone react with relatively slight degrees of anaphylaxis three weeks after sensitization. When the insulin was combined with horse serum fair grades of anaphylaxis were obtained at the three weeks shock (Table I). However, on the second shock three weeks after the first intravenous assay, those animals which had received insulin alone in initial sensitization showed anaphylactic reactions at least equal to and generally greater than those in the horse serum-insulin group had shown at the three week shock (Tables I and II). No experiments were made to bring out differences in the latter group at a second three week shock.

The possible combinations of factors in this experiment suggested that the variations in the type of insulin used, the time, the number and the route of injections might be of importance. Accordingly further experiments designed

TABLE I

THE DEGREE OF ANAPHYLACTIC RESPONSE WITH COMMERCIAL INSULIN, AFTER SENSITIZATION WITH INSULIN ALONE AND WHEN "COMBINED" WITH HORSE SERUM

GROUP	ANIMAL NO.	FIRST INTRAVENOUS SHOCK AT THREE WEEKS		SECOND SHOCK INTRAVENOUSLY THREE WEEKS LATER	
		TYPE INSULIN AND UNITS	REACTION	TYPE INSULIN AND UNITS	REACTION
Horse serum plus insulin 0.4 c.c. subcut. 3 doses	a	Commercial insulin 8 units	3+	Horse serum 0.2 c.c.	
	b		1+		
	c		4+		
	d		1+		
	e		4+		
	f		4+		
	g		1+		
	h	Commercial insulin 8 units	—	Commercial insulin 8 units	—
	i		1+		4+
	j		0		4+
	k		4+		
Commercial insulin 0.2 c.c. subcut. 3 doses	l	Commercial insulin 8 units	4+		
	m		2+		
	n		0		
	o		—		4+
	p		—		3+
	q		—		4+

Anaphylactic Response

4+ = lethal, with jactitation, convulsions, etc.

3+ = dyspnea, convulsions, prostrations with survival

2+ = dyspnea, cyanosis, wheezing, cough, loss of posture

1+ = cough, sneeze, scratching of nose, cyanosis, slight dyspnea, micturition, defecation, nervousness

TABLE II

THE DEGREE OF ANAPHYLACTIC RESPONSE IN GUINEA PIGS "SENSITIZED" WITH CRYSTALLINE INSULIN

GROUP	ANIMAL NO.	FIRST INTRAVENOUS SHOCK AT THREE WEEKS		SECOND SHOCK INTRAVENOUSLY THREE WEEKS LATER	
		TYPE INSULIN AND UNITS	REACTION	TYPE INSULIN AND UNITS	REACTION
A1 u 2-4-6 Subcut.	50	Crystalline insulin 8 units	—	10	4+
	55		—	10	—
	58		—	10	4+
	60		—	19	4+
	67		—	19	4+
	49	Commercial insulin 8 units	—	10	3+
	70		—	15	—
	66		—	19	2+
	56		—	Commercial insulin 19	0
	73		—	19	2+
A2 u 6-4-4 Subcut.	1	Crystalline insulin 4 units	0	Crystalline insulin 4 units	4+
	3		2+		—
	7		1+		4+
	8		4+		—
B u 6-4-4 Intrav.	17	Crystalline insulin 4 units	1+	Crystalline insulin 4 units	—
	18		3+		—
	19		0		4+
	20		0		0
	21		1+		—
	22		0		0
	23		2+		4+
	24		2+		1+

to demonstrate possible differences were carried out and are included in the tables (groups B, Tables II and III, and Table IV). Both crystalline and

TABLE III

THE DEGREE OF ANAPHYLACTIC RESPONSE IN GUINEA PIGS SENSITIZED WITH COMMERCIAL INSULIN

GROUP	ANIMAL NO	FIRST INTRAVENOUS SHOCK AT THREE WEEKS		SECOND SHOCK INTRAVENOUSLY THREE WEEKS LATER	
		TYPE INSULIN AND UNITS	REACTION	TYPE INSULIN AND UNITS	REACTION
A1 u 246 Subcut	52	Commercial insulin 8 units	—	Commercial insulin 19 units	—
	53		—		0
	63		—		0
	65		—		1+
	74	Crystalline insulin 8 units	—	Crystalline insulin 19 units	—
	51		—		4+
	57		—		0
	59		—		0
	68		—		0
	61		—		0
A2 u 644 Subcut	71	Commercial 8 units	—	Crystalline insulin 19 units	3+
	69		—		4+
	26	Commercial insulin 4 units	0	Commercial insulin 4 units	2+
	27		—		4+
	28		0		—
	29		0		—
	30		2+		1+
	32		4+		—
B u 644 Intrav	41	Commercial insulin 4 units	0	Commercial insulin 4 units	—
	42		0		—
	43		1+		0
	44		2+		1+
	45		0		2+
	46		0		1+
	47		0		2+
	48		1+		2+

commercial insulin were used in every series. The animals shown in Table IV were "shocked" three weeks after the last sensitizing dose by a subcutaneous injection, and three weeks later by the intravenous route. The subcutaneous

TABLE IV

THE DEGREE OF ANAPHYLACTIC RESPONSE IN ANIMALS "SHOCKED" BY SUBCUTANEOUS ROUTE AND THREE WEEKS LATER BY INTRAVENOUS ROUTE

GROUP	ANIMAL NO	FIRST SHOCK SUBCUTANEOUSLY AT THREE WEEKS		SECOND SHOCK INTRAVENOUSLY THREE WEEKS LATER	
		TYPE INSULIN AND UNITS	REACTION	TYPE INSULIN AND UNITS	REACTION
Crystalline insulin u 644 Subcut	10	Crystalline insulin 4 units	0	Crystalline insulin 4 units	2+
	11		0		4+
	13		0		—
	16		—		4+
	33	Commercial insulin 4 units	0	Commercial insulin 4 units	4+
	34		0		0
	35		0		—
	37		0		3+
	38		0		0
	39		0		—
	40		0		0

"shocking" was insignificant and apparently did not alter the grade of response at the second shock three weeks thereafter by the usual intravenous method. This would indicate that time rather than a previous intravenous injection of insulin is the factor of importance in establishing anaphylactic sensitization in these guinea pigs. Both groups B (Tables II and III) were given all 3 preparatory doses by intravenous routes, as well as the shock dose. These animals showed moderate but definite anaphylaxis at the first three-week assay but not significantly different from those sensitized by subcutaneous injections (groups A2, Tables II and III). At six weeks there were likewise no notable differences, though the crystalline insulin groups showed slightly more marked reactions. Within a given group the animals varied somewhat in their individual responses, but these variations from the experimental result were neither greater nor more frequent than is seen in any anaphylactic study in guinea pigs. Certain of the differences in the height of the reaction which at first glance seem inconsistent, are readily accounted for by differences in the unitage of the shock dose employed. The demonstration of precipitins for insulin was inconclusive. No Dale preparations were studied in this experiment.* Observations on skin tests with insulin in sensitized animals will be reported at a later date.

COMMENT

The ability successfully to sensitize guinea pigs with insulin as demonstrated in this experiment is not surprising. Jensen and others¹¹⁻¹⁵ have demonstrated that insulin is a complete protein and is without organ or species specificity; accordingly, we might reasonably expect it to exhibit the immunologic properties of a protein. The slightly greater anaphylactogenic potency of crystalline insulin has been mentioned above. It is possible that this variation may be intimately related to the relative differences in purification. Sulfur in the insulin molecule has been shown to be labile. The possible internal rearrangements which might take place in the insulin molecule in the presence of "foreign" material, such as specific serum and tissue products of the host of origin, are purely conjectural. Such rearrangements, however, might conceivably alter or modify its action. Efforts to control the route of sensitization and the factor of time disclosed that the use of the intravenous route made no appreciable difference in the degree of shock at the first three-week assay. It is seen, however, that the combination of insulin with horse serum produces a fairly consistent and moderate grade of anaphylaxis after three weeks (Table I).

Of considerable interest is the relative absence of fatal hypoglycemic shock from the insulin dosage used. The possible explanations might be: (a) During the sensitization the animals were not fasting, and were returned to cages where abundant food awaited them. (b) The combination of insulin with guinea pig serum might alter the potency of the insulin so as to reduce its effect (this is discussed elsewhere).¹⁶ (c) In some instances liberal quantities of intraperitoneal glucose were used either to combat or forestall hypoglycemic shock.

*Since the completion of this work a study of the antigenic properties of insulin has been reported by J. H. Lewis.¹⁷ By employing the Schultz-Dale technique, utilizing strips of uterus taken from virgin guinea pigs previously injected subcutaneously with 1 mg. doses of beef and pork insulin, he demonstrated a definite anaphylactic response. He thus showed that sensitization rested not on the proteins of the animal of origin (of the insulin) but apparently upon the insulin molecule itself.

(d) In guinea pigs showing no hypoglycemia and not given glucose the question of the antihormone effect of Collip¹ and others arises. However, reasonable doubts have been cast on the existence of antihormones by the work of Sulman with the gonadotropic substance¹⁵. A similar end result which may be indistinguishable from the so called antihormone effect may theoretically be due to specific antibodies to the endocrine substance which unite with the antigen and thereby impede its action. Such antibodies have not been conclusively demonstrated and the explanation of this phenomenon remains to be elucidated.

Of practical significance is the fact that sensitization to insulin has been produced in the guinea pig. In the human being this sensitizing property of insulin might be seen to be more in evidence on resumption of therapy after a period of insulin freedom, which permits the sensitization to manifest itself, whereas such sensitization had been previously obscured by the frequent therapeutic (and incidentally desensitizing) injections. However, follow up data from cases known to have received insulin intermittently would have to be studied to affirm or deny the validity of such a theory. Whether the extended use of crystalline and protamine insulin will produce more frequent or less frequent sensitizations to insulin remains to be seen.

SUMMARY AND CONCLUSIONS

1 Guinea pigs injected parenterally with commercial and crystalline insulin reacted with anaphylaxis on subsequent intravenous injection.

2 Guinea pigs sensitized by subcutaneous or intravenous injections of insulin reacted with varying degrees of anaphylactic shock at intervals of three and six weeks following sensitization. The first shock at three weeks was generally less severe than the second at six weeks using the same shock dose of insulin.

3 When the guinea pigs were sensitized with a combination of horse serum and insulin, fair degrees of anaphylaxis to insulin were obtained from a shocking dose three weeks after the last sensitizing injection.

4 The questions of insulin resistance and insulin hypersensitivity are briefly considered.

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THE RELATIONSHIP OF INSULIN HYPOGLYCEMIA TO THE METHOD OF ADMINISTRATION AND THE TYPE OF INSULIN*

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THE recent discovery of protamine insulin and the increased availability of crystalline insulin have given origin to many reports concerning variations in their hypoglycemogenic effects. In a study of certain of the immunologic features of insulin, incidental observations were made which were considered worthy of record. For example, it was noted that those guinea pigs injected subcutaneously developed typical hypoglycemic reactions in one and one-half hours to two hours, whereas those receiving insulin (commercial or crystalline) intravenously during the sensitization period did not exhibit this phenomenon. The discrepancy was not easy to explain since the animals were treated under identical conditions and with the same insulin dosage. This observation led us to investigate the possible mechanism of such differences. The likelihood of a prompt combination of the injected insulin with the circulating blood suggested that a not too remote parallel might be obtained by a mixture in vitro of serum and insulin prior to injection. A study of the blood sugar responses following the injection of such a mixture and of other standard preparations of insulin was made and constitutes the basis of the following report.

EXPERIMENTAL

Materials and Methods.—Seven rabbits, average weight 3 kg., were used for the assays, the great majority of the determinations being done, however, on 4 rabbits. Each rabbit was injected at different times with 10 unit doses of the various insulin preparations recorded below by subcutaneous and intravenous routes. An interval of at least forty-eight hours elapsed between injections in any one animal. Some of the rabbits were used for 14 separate studies, and 2 animals were used only twice. A minimum of five studies was made in the

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case of commercial insulin intravenously and the maximum of fourteen was completed with commercial insulin subcutaneously. The number of experiments with any one preparation was determined largely by the consistency with which the curves could be reproduced. Those preparations which gave rather variable results were subjected to more determinations in order to arrive at a significant average level, whereas consistently similar responses on every assay obviated the necessity of more numerous determinations. Blood was drawn from the ear vein by a micropipette at thirty minute intervals for blood sugar determinations by the micro method of Hagedorn and Jensen.² The animals were interchanged as to types of insulin employed. Each period of observation lasted two and one half to three hours, unless interrupted by hypoglycemic reactions requiring supplementary glucose. Equal parts of insulin (10 units) and guinea pig serum were mixed in a sterile test tube and allowed to stand at room temperature for several hours until used. No precipitate was noted. This procedure was carried out with both the commercial and crystalline insulins. Guinea pig blood was obtained by cardiac puncture, and after separation of the clot the serum was drawn off and stored in the refrigerator for use as required. Fresh serum was obtained at frequent intervals. The insulin serum mixtures were prepared before each new series was run.

The insulin was obtained from several sources each of which had previously standardized the preparation according to the method set forth in the New and Non Official Remedies. These brands of insulin are in common use everywhere and for that reason were not standardized by us. Dr. H. Jensen, of the Johns Hopkins University School of Medicine, kindly supplied us with the powdered crystalline insulin used in the early studies. One milligram of this insulin represents the equivalent of 24 units and the solution was prepared by dissolving the calculated amount in 0.001 hydrochloric acid so that 0.1 cc. of the solution contained one unit of insulin. We are indebted to E. R. Squibb and Sons, who, through Dr. John E. Anderson, gave us two purified preparations of commercial insulin derived from beef pancreas.

- (1) unit content = 223 U per cc.
nitrogen content = 0.0075 to 0.01 mg. per cc.
- (2) unit content = 40 U per cc.
nitrogen content = 0.0076 mg. per cc.

The Frederick Stearns Company generously supplied a quantity of crystalline insulin, 40 units per cc., with a nitrogen content of 14 per cent (dry crystals) and assay approximately 24 to 25 units per milligram. The results are based upon 64 experiments comprising 269 separate blood sugar determinations and are expressed as the percentage drop in blood sugar using 100 per cent as the fasting level (see Table I). Studies on protamine insulin were not made.*

TABLE I

THE AVERAGE LEVEL OF BLOOD SUGAR AT VARIOUS INTERVALS IN THE EXPERIMENT EXPRESSED IN TERMS OF PERCENTAGE OF THE ORIGINAL BLOOD SUGAR

TIME (MINUTES)	COM- MERCIAL INSULIN 10 U SUBCUT	COM- MERCIAL INSULIN 10 U INTRAV	COM- MERCIAL INSULIN 10 U PLUS SERUM SUBCUT	CRYSTAL- LINE INSULIN 10 U SUBCUT	CRYSTAL LINE INSULIN 10 U INTRAV	CRYSTAL LINE INSULIN 10 U PLUS SERUM SUBCUT
38	40.12	42.95	35.67	44.35	48.01	30.35
76	24.71	50.06	39.15	53.03	69.42	33.81
112	49.20	67.54	46.48	49.95	53.97	42.47
148	47.12	56.18	41.08	48.84	55.18	37.10
218	54.35	38.30			43.80	
Average per cent drop per experimental period	46.70	51.006	40.095	49.042	54.074	35.932

*Since the completion of this work H. Blotner has reported a similar study on protamine insulin in which he found the hypoglycemic potency of this preparation increased by the addition of serum.

RESULTS

Objectively, commercial and crystalline insulin produced definite hypoglycemic effects within one to two hours. The crystalline preparation was in general more effective. Commercial insulin plus guinea pig serum likewise elicited unmistakable insulin reactions. Crystalline insulin plus guinea pig serum produced the least number of reactions. In decreasing order of hypoglycemic potency were:

(1) Crystalline insulin intravenously, (2) commercial insulin intravenously, (3) crystalline insulin subcutaneously, (4) commercial insulin subcutaneously, (5) commercial insulin plus serum subcutaneously, and (6) crystalline insulin plus serum subcutaneously. (Insulin plus serum intravenously was not studied in this experiment.)

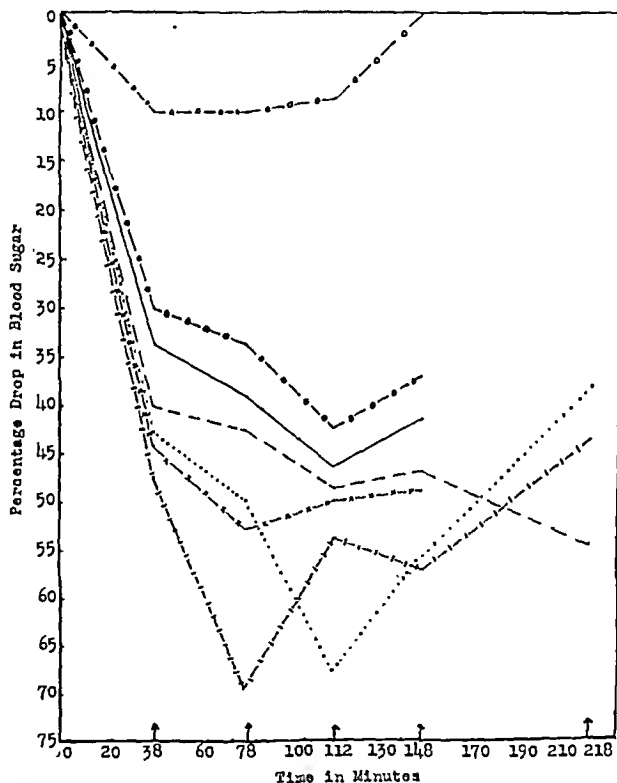


Chart 1.—Hypoglycemic effect (in the rabbit) of commercial and crystalline insulin alone and when "combined" with guinea pig serum, computed as a percentage drop in blood sugar.

- Guinea pig serum subcutaneously.
- Crystalline insulin plus serum subcutaneously.
- ×—×—×— Commercial insulin plus serum subcutaneously.
- — — — Commercial insulin subcutaneously.
- ×—×—×— Crystalline insulin subcutaneously.
- — — — Commercial insulin intravenously.
- |—|—|— Crystalline insulin intravenously.

Several points of interest can be gathered from the chart of the blood sugar curves (Chart 1), obtained by averaging the findings in the various groups of studies. The most rapid drop occurs after intravenous injection of crystalline insulin which is maximal at an average of about seventy-eight minutes after injection. Commercial insulin intravenously acts only slightly

more slowly, reaching its maximum "low" at an average of one hundred and twelve minutes. This is in distinct contrast to the previous clinical reports (on subcutaneous injection^{2,3}). The curves for subcutaneous crystalline and commercial insulin show that by subcutaneous route crystalline insulin is still, albeit slightly, more hypoglycemic than commercial. The "combined" insulin serum preparations showed the least effect. Both were still distinctly hypoglycemic, the activity of crystalline insulin being slightly more reduced by such combination. The effect of the manipulative procedure with out insulin was studied as a control, and no appreciable alterations in blood sugar were noted. Guinea pig serum used alone as an additional control caused a slight drop in the blood sugar level (see Chart 1) without observable reaction.

It was interesting to note the blood sugar level at which convulsions appeared (see Chart 2). The average level was about 35 mg per cent, with the majority of convulsions appearing when the blood sugar ranged from 30 to 50 mg per cent. Occasionally blood sugar levels within and below this range were not accompanied by convulsions. In two instances, reactions appeared

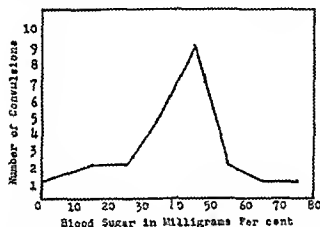


Chart 2—The blood sugar level at which 24 observed insulin convulsions appeared in the rabbit

when the blood sugar was 73 and 66 mg per cent, respectively. Several observers⁴ have felt that the convulsive level in the usual rabbit assay is not uniform and that the appearance of convulsions does not necessarily coincide with a reduction of the blood sugar to 45 mg per cent. It is not at all unlikely that of equal importance to the absolute level is the gradient, or rate of drop, of the blood sugar. The above results, however, agree fairly closely with the convulsive range of 50 to 26 mg per cent obtained by Dotti.¹

COMMENT

It is of considerable interest that, in contrast to the earlier reports in human beings in which crystalline insulin given subcutaneously was shown to have a slower but longer hypoglycemic effect, we find that in the rabbit the effect of crystalline insulin is not only in general more prompt but of less duration than the commercial preparation. This is also counter to our observations in guinea pigs that the intravenous injection of crystalline and commercial insulin was less likely to produce clinical hypoglycemia. In the rabbit the blood sugar curves show a greater drop with intravenous crystalline insulin than with any other preparation. Whether this may be explained on

the basis of a more gradual gradient in the drop in blood sugar, albeit to a lower level, or whether there is a true species difference between guinea pigs and rabbits in their reactions to insulin is not clear. An ever-present hazard in interpretation is the possible discrepancy in the unit assay by the manufacturers of the various types of insulin used. Since their methods are standard and their products in general clinical use, it was felt that re-assay would be unnecessary, particularly in view of the fact that these data were gathered secondarily to an investigation of the immunologic properties of insulin.

The reduced effect of insulin combined with serum also raises several questions. These have to do primarily with the mechanism of this reduction. Is there an "antigen-antibody" union which inactivates insulin? Does insulin combine with the constituents of the serum to reduce its hypoglycemic effect? Is there a so-called "antihormone"?¹⁰ Or is insulin potency lost through enzymatic breakdown? It has been reported that normal human blood destroys the effect of crystalline insulin after incubation for several days at 37° C. Blood corpuscle-free serum has a similar though less intense effect. Inactivation of the blood serum by heating at 56° C. nullifies this destructive effect. Leucocyte-rich blood from leucemias has a more destructive action on insulin than normal blood. Similar studies have been made with blood from diabetic patients. Mauriac and Aubertin,⁹ and Karelitz and his co-workers¹⁰ have reported that diabetic plasma neutralizes insulin *in vitro* to a greater extent than does normal plasma. They felt that this neutralizing substance was trypsin. Black,¹¹ on the other hand, could not confirm these observations. Possibly trypsin forms inactive adsorption complexes with insulin; this undoubtedly occurs *in vitro* in strongly acid solutions.¹² Inactivation of insulin has also been accomplished by removing part of its sulfur content;¹³ this is not too remote a possibility since Jensen and his associates¹⁴ have shown that the hypoglycemic property of insulin is associated with certain di-thio (-S-S-) groupings present, in part at least, as combined cystine. This labile sulfur may unite in some way through molecular rearrangement with the serum with which it is mixed so as to alter appreciably the hypoglycemic effect. The existence of an anti-insulin^{15, 16} has been hypothesized by several investigators, but as yet the existence of antihormones is without corroborated experimental foundation.¹⁷ Experiments are now in progress by which some of the obscurity concerning this phenomenon may be clarified. With it are perhaps bound up some of the more bewildering phases of so-called insulin resistance. As a method of attack on the problem it is not without some promise.

SUMMARY AND CONCLUSIONS

1. The effects of commercial and crystalline preparations of insulin on the blood sugar of the rabbit were studied. Comparisons were made between the effects of subcutaneous and intravenous injection.

2. Crystalline insulin in the rabbit produced a greater drop in the blood sugar and one less sustained than did commercial insulin. This difference was greater on intravenous than on subcutaneous administration.

3 Guinea pig serum in combination with each type of insulin reduced the rate of hypoglycemiogenesis but, unlike protamine insulin (current reports), did not substantially prolong it. Crystalline insulin suffered the greater reduction in potency.

4 The theoretical implications of the work are discussed.

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A COMPARISON OF THE ANTIPERNICIOUS ANEMIA POTENCY OF DEPEPSINIZED AND UNDEPEPSINIZED GASTRIC MUCOSA*

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IN ANOTHER communication¹ we are reporting some of our studies on pernicious anemia including an inquiry into the rôle of pepsin. We observed that depepsinized whole stomach mucosa, depepsinized fundus mucosa, and depepsinized pylorus mucosa, obtained from the hog's stomach under proper conditions for preserving antipernicious anemia potency and prepared according to the Greenspon process were all practically inactive in the treatment of

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pernicious anemia when sources of extrinsic factor were excluded from the diet. When extrinsic factor was added there was minimal evidence of antipernicious activity in the patient fed with adequate amounts of depepsinized pyloric mucosa, but not in the patients fed with similar doses of depepsinized whole stomach or depepsinized fundus mucosa.

Since Greenspon² had claimed a higher activation of antipernicious anemia potency by depepsinization, we suggested¹ that the impairment of intestinal permeability may have accounted for our inability to confirm his conclusions. Moreover, we suggested that the process of depepsinization may not always result in the same end product and that whereas in one instance an active product may be obtained, in another the product may be inactive.

It occurred to us that the inactivity of our depepsinized products was so striking that another possible explanation seemed equally, and perhaps more, plausible than those just mentioned. In other words, though we permitted ourselves to question quite reasonably the degree of intestinal permeability, we could not doubt the adequacy of our sources of extrinsic factor. The possibility seemed apparent, therefore, that the process of depepsinization could have inactivated the intrinsic factor inasmuch as the response to products of known potency, though submaximal, was definite enough to rule out the factor of intestinal impermeability. We, therefore, decided to observe a patient with pernicious anemia treated with whole stomach material prepared as follows: The material was obtained from the slaughter house and preserved in dry ice until brought to the laboratory, when it was ground and immediately placed in a vacuum dryer, in which the temperature at no time exceeded +40° C. At the end of about thirty-six to forty-eight hours, it was further dried to remove the last traces of moisture over phosphorus pentoxide and then defatted with petroleum ether. In other words, the material differs essentially from the Greenspon process in that it is simply desiccated dried gastric mucosa without any attempt being made to remove the pepsin or to go through the various extractions as described by Greenspon.

If such a product were potent then we could assume that the process of depepsinization destroyed rather than enhanced the potency of these preparations. It is important to keep clearly in mind that even if the process of depepsinization inactivated the antipernicious anemia potency of these preparations that result does not necessarily mean that the absence of pepsin per se decreases the potency. In other words, a distinction must be made between the physicochemical changes occurring during depepsinization and the theoretic removal of pepsin without subjecting the mucosa to any changes which might influence the antipernicious anemia potency.

With these facts in mind we proceeded to treat a patient with typical pernicious anemia with undepepsinized desiccated whole stomach mucosa (see Table I).

The data in Table I (Patient J. L., aged 48 years) show conclusively that this preparation is completely satisfactory as a source of intrinsic factor. When compared to the responses of patients treated with depepsinized gastric mucosa (whole stomach, pylorus, fundus) as previously reported,¹ it would appear that Greenspon's process in some way results in loss

TABLE I

EFFECT OF ORAL ADMINISTRATION OF UNDEPEPSINIZED WHOLE STOMACH MUCOSA
DIET CONTAINS EXTRINSIC FACTOR DESICCATED GASTRIC MUCOSA HAS
ANTIPERNICIOUS ANEMIA POTENCY

DAYS OF TREATMENT	RBC MILLIONS	HB GM	PER CENT	RETICULO- CYTES PER CENT	TREATMENT DIET EXCLUDING INTRINSIC FACTOR AND INCLUDING EX- TRINSIC FACTOR *
1	245	79			"
2	260	89	60	25	"
3				22	"
4				30	"
5	270	86		19	"
6				15	" 24 gm undepepsin- ized whole gastric mucosa q d
7				31	" " "
8	26	95		42	" " "
9				37	" " "
10				74	" " "
11	31	110		88	" " "
12				109	" " "
13				124	" " "
14	355	99		53	" " "
15				87	" " "
16	355	110		"	" " "
17				44	" Liver extract 5 cc in transmucosally
18				24	" " "
19					" " "
20					" " "
21	325	106			" " "
22					" " "
23	305	112		15	" " "
24					" " "
25					" " "
26				20	" " "
27	36	117			" " "

*The extrinsic factor was provided in the form of meat and Brewer's yeast

of activity of the gastric mucosa. However we decided to repeat the administration of depepsinized preparations in increased dosage to two other patients in order to be sure that the maximum dosage of 40 gm each day, as already reported, was sufficient. In the two following tables are recorded the data obtained when each of the two patients was fed with 50 gm of depepsinized material each day. Both patients were ideal for the experiment not only because their counts were low but also because they represented typical uncomplicated cases (no neurologic signs or symptoms) of pernicious anemia. In view of the very low red blood count and hemoglobin readings we were advised to administer 30 gm of our undepepsinized materials daily in order to be certain to obtain satisfactory responses. The equivalent dosage of ventriculin would be about 40 gm daily. Our decision, therefore, to administer 50 gm each day would insure adequate dosage.

Table II (Patient W. W., aged 63 years) — This patient's blood did not respond to adequate dosage of depepsinized pylorus mucosa administered orally. Oral and intramuscular therapy of known potency produced a characteristic response.

Table III (Patient S. M., aged 65 years) — Curiously enough, the depepsinized fundus material given in the dosage tabulated above was followed by a reticulocyte response of 7 per cent on the sixth day after the onset of treatment. Whether this response, in the light of our previous data, was due to depepsinized fundus mucosa or to some extraneous factor or was spontaneous, it is difficult to be sure. The expected maximum reticulocyte percentage after desiccated stomach given orally is about 40 per cent. The response in the patient above is distinctly minimal.

TABLE II

EFFECT OF ORAL ADMINISTRATION OF DEPEPSINIZED PYLORUS MUCOSA. DIET CONTAINS EXTRINSIC FACTOR

DAYS OF TREATMENT	R.B.C. MILLIONS	HB. PER CENT	RETICULOCYTES PER CENT	TREATMENT
1	1.18	28	0.5	
2	1.15	28	0.5	Blood transfusion of 350 c.c. citrated blood*
3	1.50	34	0.0	House diet excluding liver, sweet-breads, and kidneys.
4				50 gm. depepsinized pylorus q. d.
5	1.25	32	0.0	"
6	1.37	35	1.3	"
7	1.20	30	1.0	"
8				"
9	1.80	36	0.4	"
10	1.61	29	0.4	"
11	1.75	32	0.2	"
12				"
13	1.50	30	0.3	"
14				Extralain tabs. 5 t. i. d.
				Dextron cap. 2 t. i. d.
				Liver extract 2 c.c. intramuscularly
15				"
16	1.64	30	0.3	"
17				"
18				"
19				"
20				P. A. diet
21	1.50	30	13.2	"
22				"
23	2.06	40	19.3	"
24	2.20	40	7.9	"
25				"
26				"
27	2.20	40 (6.4) gm.	4.5	"
34	3.23	54 (8.6) gm.	0.4	"
38	3.68	60 (9.6) gm.	1.1	"

*The transfusion was given before it could be countermanded. However, it is apparent from the data that this procedure did not influence the response.

TABLE III

EFFECT OF ORAL ADMINISTRATION OF DEPEPSINIZED FUNDUS MUCOSA. DIET CONTAINS EXTRINSIC FACTOR

DAYS OF TREATMENT	R.B.C.	HB. PER CENT	RETICULOCYTE PER CENT	TREATMENT
1				25 gm. depep. fundus. Admitted late in evening
2	1,100,000	29	0.5	50 gm. depep. fundus
3				" " " "
4	820,000	31	3.5	" " " "
5				" " " "
6	1,020,000	30	7.0	" " " "
7				" " " "
8	980,000	26	5.0	" " " "
9				" " " "
10	1,020,000	28	4.0	25 gm. depep. fundus*
11	870,000	26	4.5	" " " "
				Transfusion given and intramuscular liver begun

*Patient reported severe nausea and inability to continue medication.

SUMMARY

The reticulocyte response to undepepsinized whole stomach administered orally conformed to the expected maximal as reported by Bethell and Goldhamer.³ There was no reticulocyte response to depepsinized pylorus mucosa administered orally. In a previous report we found a minimal reticulocyte response but, on the other hand, there was no evidence in that report of antipernicious anemia activity in the fundus material. In the present instance (see Table III) there seemed to be some potency in the depepsinized fundus material administered. This response could however, be due to the greater dosage of the substance administered or to the severity of the degree of the anemia or to the possibility, previously suggested, that the process of depepsinization may not always result in the same end product. However since this patient had been active and remarkably free of distressing symptoms it seems likely that he must have been fortunate enough to have periodic, though very mild, spontaneous reticulocyte reactions, that is minimal remissions.

Since we had already had occasion to treat two patients with pernicious anemia with depepsinized whole stomach without obtaining sufficient evidence of the presence of antipernicious anemia potency in it, we considered those cases a part of the present study in order to make the investigation complete.

The patients reported in this study were typical instances of pernicious anemia. There were no neurologic symptoms or signs. Achylia was present in each instance.

CONCLUSION

Cases of pernicious anemia treated with orally administered depepsinized gastric mucosa prepared by Greenspon's process show almost completely negative results. On the other hand, desiccated gastric mucosa, administered orally, produces the calculated maximum reticulocyte response. Therefore, it would appear that the process of depepsinization inactivates the antipernicious anemia activity of gastric mucosa. In other words it apparently destroys the intrinsic factor since extrinsic factor had been provided in adequate amounts and the factor of intestinal permeability sufficiently accounted for. The exact rôle, however, of pepsin in pernicious anemia still remains a problem for further study inasmuch as our investigation has only proved that the process of depepsinization is responsible for the destruction of antipernicious anemia activity of these preparations.

NOTE. To Dr. John F. Anderson, Director, Biological Laboratories, F. R. Squibb & Sons. I owe my thanks for his cooperation in supplying me with the materials used in these studies. The expenses of this investigation were defrayed in part by a grant from the Julius Friedenwald Research Fund of the University of Maryland School of Medicine.

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LABORATORY METHODS

THE IDE TEST FOR SYPHILIS*

A COMPARATIVE STUDY WITH THE KLINE HEATED SERUM TESTS AND COMPLEMENT FIXATION METHODS, WITH CLINICAL EVALUATION IN MORE THAN 1,000 PATIENTS

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IN A RECENT publication¹ Ide described a new coloring method for the serologic diagnosis of syphilis. The present paper is a report on a comparative study of this test with the Kline heated serum tests and the complement fixation methods with clinical evaluation in more than one thousand cases. The blood specimens used in this study were collected from patients in a private dermatological practice and those attending skin clinics. An additional two hundred specimens were obtained from routine hospital patients.

The details of the test, as originally described by Ide, are as follows:

Materials.—1. Raw serum or whole finger blood.

2. Diluted Ide antigen. This is prepared by mixing 0.2 c.c. of Ide antigen with 0.6 c.c. of 2.5 per cent solution of sodium chloride in a small test tube. This mixture is shaken about thirty times.

3. Specially made hollow glass slides, having three depressions each, with a diameter of about 2 cm. and a depth of 2 mm., and a glass cover large enough to cover the entire slide.

4. Special dropping pipettes, so gauged that they deliver 0.03 c.c. of diluted Ide antigen, and others to deliver 0.03 c.c. of serum.

Technique.—1. Place 0.03 c.c. of raw blood serum in one cavity of the special hollow glass slide and spread out evenly over entire surface of depression.

2. Add one drop of diluted Ide antigen directly on to the serum.

3. Rotate the slide vigorously on a flat surface forward and backward as well as sideways for three or four minutes to insure adequate mixture. (In a later communication, Ide recommends prolonging the shaking time to eight minutes.)

4. Examine the results through a microscope with the low power. A positive reaction is evidenced by the appearance of purplish colored clumps, while in the negative reaction only tiny purplish bodies are seen.

Although Ide advocated the routine use of raw sera, he states that such sera obtained from syphilitic patients with varying amounts of treatment may occa-

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sionally give a negative reaction. In such instances he suggests heating the sera for twenty minutes. In a preliminary study the Ide test was done with 26 raw sera. These sera were then inactivated at 56° C for thirty minutes, and the Ide test repeated. It was found that the inactivated sera gave results which were as specific and considerably more sensitive (see Protocol 1). In this study, therefore, all the sera for the Ide tests were inactivated at 56° C for thirty minutes.

PROTOCOL 1

COMPARISON BETWEEN 26 RAW AND HEATED SERA IDE TESTS

RAW SERUM IDE	HEATED SERUM IDE
±	++++
++	+++
+++	++++
-	++++
-	++++
-	++
-	-
+	++++
+	+
-	+
++++	++++
-	+
-	-
-	+
±	++++
++++	++++
+	++
-	-
++++	++++
-	-
-	-

Furthermore, instead of employing the specially made hollow glass slides advocated by Ide, there were utilized the paraffin ring slides described by Green² for the Kline heated serum test.³ These slides measure 2 by 3 inches, on which are mounted twelve paraffin rings each with an inside diameter of 14 mm. This enables the serologist to do twelve tests on each slide. Since Ide advocated the preparation of 0.08 c.c. of diluted antigen which is sufficient for thirty tests, and is satisfactory for use for thirty minutes, the serologist can do these 30 tests on three Kline test slides at one time. In addition, by using the Kline test slide instead of the hollow glass Ide slide the tendency to evaporation is minimized.

Early in this study three hundred sera were tested on the Ide and Kline test slides with identical results. It was also found that with the Kline test slide as well as the Ide slide, the optimal rotation time was eight minutes.

The modified technique of the Ide test as used in this study is as follows:

1. Pipette 0.03 c.c. of inactivated serum into each paraffin ring.
2. Add one drop of diluted Ide antigen (approximately 0.03 c.c.)
3. Rotate the slides with moderate vigor for eight minutes.

4. Read degree of clumping through microscope (low power 16 mm. objective, eyepiece 12) at a magnification of about 120 times, with the light cut down as for the study of urinary sediments, and reported in terms of pluses according to the degree of clumping and the size of the clumps.

RESULTS

Comparison of 1,037 Ide and Kline Heated Serum Tests.—For this comparative study, blood specimens were obtained from 1,037 patients by venepuncture, and the sera heated at 56° C. for thirty minutes. The patients were divided into syphilitic and nonsyphilitic groups (see Table I). The former group consisted of 555 patients who either presented definite syphilitic lesions, or who admitted a previous syphilitic infection, or who were receiving antisyphilitic treatment at the time of this study.

TABLE I

COMPARISON OF IDE TEST AND KLINE DIAGNOSTIC TEST WITH 1037 SERA

SYPHILITIC GROUP (555 SERA)					NONSYPHILITIC GROUP (482 SERA)				
AGREEMENT		DISAGREEMENT			AGREEMENT		DISAGREEMENT		
AB-SOLUTE	RELATIVE	POSITIVE KLINE DIAGNOSTIC NEGATIVE IDE	NEGATIVE KLINE DIAGNOSTIC POSITIVE IDE		AB-SOLUTE	RELATIVE	POSITIVE KLINE DIAGNOSTIC NEGATIVE IDE	NEGATIVE KLINE DIAGNOSTIC POSITIVE IDE	
Tests	436	103	14	2	Tests	444	37	0	1
Per cent	78.55	18.56	2.52	0.36	Per cent	92.11	7.67	0	0.21

The nonsyphilitic group consisted of 482 patients who presented no clinical or serologic evidence of syphilis. The sera of all the patients in this group gave negative results with both the Kline diagnostic and exclusion heated serum tests. The specificity and sensitivity of the Kline tests have already been established by the recent serologic conferences^{4, 5} held in this country, under the auspices of the United States Public Health Service in cooperation with the American Society of Clinical Pathologists. The details of the Kline diagnostic and exclusion heated sera tests are given in Kline's book.³

The evaluation of the results of this comparative study was made according to the method of Kahn as follows: positive reactions, four-plus, three-plus, and two-plus; doubtful reactions, one-plus and plus-minus; agreement, positive or negative by both methods; relative agreement, positive or negative by one method and doubtful with the other; disagreement, positive by one method and negative with the other.

Of 482 nonsyphilitic persons whose sera gave negative reactions with the Kline diagnostic and exclusion test, 444 (92.11 per cent) gave negative reactions with the Ide test. In this group there were 37 (7.67 per cent) false doubtfuls and one (0.21 per cent) false positive reactions with the Ide test. This demonstrates that in this selected group the Ide test is somewhat less specific than the Kline heated serum tests.

Of the 555 syphilitic sera there were 16 (2.88 per cent) disagreements, with a resulting total agreement of 97.11 per cent. Of the sixteen disagreements fourteen specimens were positive with the Kline diagnostic heated serum test and negative with the Ide test, while only two specimens gave negative reactions with the Kline diagnostic heated serum test and positive reactions with the Ide test. These two specimens, however, gave positive reactions with the Kline exclusion heated serum test. Thus if the Ide test alone had been used in this group, fourteen cases of syphilis would have been missed. This comparative study demonstrates that the Ide test is less sensitive than the Kline test.

TABLE II

COMPARISON OF IDE TEST AND COMPLEMENT FIXATION TESTS WITH 837 SERA

	AB SOLUTE	REL ATIVE	POSITIVE COMPLEMENT FIXATION NEGATIVE IDE	NEGATIVE COMPLEMENT FIXATION POSITIVE IDE		AB SOLUTE	REL ATIVE	POSITIVE COMPLEMENT FIXATION NEGATIVE IDE	NEGATIVE COMPLEMENT FIXATION POSITIVE IDE
Source A Tests	98	33	11	2	Source A Tests	200	22	0	1
Per cent	68.05	22.93	7.64	1.39	Per cent	80.69	9.86	0	0.45
Source B Tests	120	41	8	4	Source B Tests	99	7	0	1
Per cent	70.88	22.53	4.39	2.2	Per cent	92.52	6.54	0	0.94
Source C Tests	11	8	2		Source C Tests	149	10	0	1
Per cent	52.38	38.1	9.52		Per cent	93.13	6.25	0	0.62

Comparison of 837 Ide and Complement Fixation Methods—The blood specimens for this group were obtained from three sources, and the technique of the complement fixation methods for each source is as follows:

Source A—Total volume of serum used in test—0.55 cc

Test made with 0.04 and 0.02 cc serum with 2.5 units of guinea pig complement

Antigen—0.2 per cent cholesterolized alcoholic extract of beef heart

Ambocceptor—diluted according to titration

Corpuscles—sheep, diluted 1:20

Sensitized cells consist of three parts of ambocceptor and two parts of 5 per cent sheep cell suspension

Incubation—First, overnight icebox, and second, fifteen minute water bath at 37° C

Source B—Total volume of serum used in test—0.5 cc

Test made with 0.05 cc and 0.02 cc serum, with 2 units of guinea pig complement

Antigen—cholesterinized alcoholic extract of beef heart.

Amboceptor—Difco, diluted 1:1000.

Corpuseles—Sheep, diluted 1:20.

Incubation—First, one hour water-bath at 37° C., and second, fifteen minutes water-bath at 37° C.

Source C.—Test made with serum varying in amounts from 0.1 c.e. to 0.05 c.e.

Complement—guinea pig, diluted 1:10 and used in amounts varying from 0.2 c.e. to 0.3 c.e.

Antigen—two plain alcoholic extracts of beef heart. One cholesterinized alcoholic extract of beef heart.

Incubation—First, thirty minutes water-bath 37° C., then two and one-half hours icebox, second fifteen minutes water-bath 37° C.

Amboceptor—diluted according to titration and used in amounts varying from 0.05 to 0.1 c.e., depending on amount of natural amboceptor present in each patient's serum.

Corpuseles—sheep, diluted 1:20, used in 0.5 c.e. amounts.

The results (see Table II) show that the Ide test was somewhat less sensitive and less specific (with three nonspecific positive reactions) than the complement fixation methods.

COMMENT

The Ide test¹ is a simple test and easily performed. The antigen consists of an alcoholic extract (95 per cent alcohol) of beef heart muscle containing 0.2 per cent cholesterin. To 100 c.c. of this cholesterinized alcoholic extract is added 5 c.c. of a 5 per cent solution of gum benzoin, and 0.1 c.e. each of a 1 per cent alcoholic solution of crystal violet and azure II. In this study (comparison of Kline and Ide tests) the Ide test (antigen supplied by Dr. Ide) done with this crude alcoholic extract of beef heart, gave only one (0.21 per cent) false positive and 37 (7.67 per cent) false doubtful reactions with sera from 482 carefully selected presumably nonsyphilitic persons. The Ide tests performed with a commercially prepared antigen, however, gave results that were considerably less sensitive than those obtained with antigen supplied by Dr. Ide.

Ide antigen is a plain alcoholic extract antigen which contains a number of substances some of which are responsible for nonspecific reactions. Kline antigen is the acetone insoluble fraction only of alcoholic extract and contains no water, alcohol, or acetone soluble impurities.

CONCLUSIONS

1. A modified technique of the Ide test is described.
2. The modified Ide test has been found to be somewhat less sensitive and less specific than the Kline heated serum tests and complement fixation methods.

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A RAPID METHOD FOR STAINING BLOOD SMEARS

A MODIFICATION OF THE ORIGINAL WRIGHT TECHNIQUE

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THIS method is particularly useful to the general practitioner. It may, however, be employed in larger hospital laboratories where time is a factor. The stain I have used is the original Wright's stain. It may or may not be fresh, cool, or stale.

After the smear has been made, place it in a convenient position over a receptacle of distilled water or hold it in a simple thumb forceps. I, personally, fill a six ounce drinking glass three quarters full of distilled water, and rest the slide on the edges of the glass.

(1) Cover the smear entirely with the Wright solution in stock. Leave it on for about ten seconds.

(2) Ignite the stain and let it flame for about ten to twenty seconds.

(3) With a suitable object, a pencil, a small rod, or any other such article, cause the flaming slide to fall into the distilled water, or drop it from the forceps into the container of distilled water.

(4) Remove, dry, and it is ready for use.

Only the nucleated cells will stain deeply. It is of interest that the nuclear material shows very much more detail than the ones stained according to the classic procedure. The red blood cells, however, are stained poorly by this method. No attempt to study them should be made at all.

In surgical cases, where a differential blood count is called for at once, this method is probably the most rapid, and yet as efficient at least as any we have at present.

A thin to medium streaked blood smear is the best to use for this method of rapid staining.

A PRACTICAL STAIN FOR THE SPIROCHETES OF SYPHILIS AND VINCENT'S ANGINA.

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THE following method of staining *Treponema pallidum* and *Borrelia vincenti* is simple, rapid, and reliable.

Diagnosis of syphilis by *quick* staining of serum exuding from a scraped lesion, or of tissue juice drawn by needle from a deep portion of a syphilitic area is of value because *all* organisms present can be observed. The method described stains the syphilis germs in these fluids *deeply*; in cases where spirochetes are few in number and dark-field tests fail to reveal them, smears stained by this method show the organisms plainly. It also yields equally good results when applied to aspirated testis juice of rabbits that have been artificially infected with *Treponema pallidum*.

Demonstration of *Borrelia vincenti* in ulcerative inflammation of mouth and pharynx is made easy by this method of staining. The spirochetes stain as deeply as other bacteria present.

The procedure is as follows:

1. Make a direct smear of the syphilis serum or tissue juice on a clean slide. In case of Vincent's angina, spread the ulcerative material in a small loopful of water on a clean slide.
2. Air dry. Fix with heat.
3. Cover with N/20 HCl for ten seconds.
4. Wash in running water for five seconds.
5. Cover with Gram's iodine solution for five to ten seconds.
6. Wash.
7. Cover with aniline-gentian violet for five to ten seconds.
8. Wash.
9. Cover with Gram's iodine solution for five to ten seconds.
10. Wash.
11. Cover with aniline-gentian violet for five to ten seconds.
12. Wash, blot, and examine.

The following suggestions are in order: (a) Gentian violet without aniline oil may be used. (b) A very deep color can be secured by applying the iodine and gentian violet solutions a third time. In staining *T. pallidum* this procedure is often of advantage. (c) The stain is not permanent, but this does not alter the diagnostic value of the method.

A COMPARISON OF METHODS FOR THE PRESERVATION OF THE HEMOLYTIC ACTIVITY OF GUINEA PIG COMPLEMENT*

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THREE or more guinea pigs are usually bled to obtain a uniformly satisfactory complement. Often some of this fresh complement is left over, and unless special methods for preservation are used the serum rapidly deteriorates. The use of suitable methods of preservation eliminates the necessity of bleeding animals each time complement is needed and allows utilization of guinea pigs for serum at times when large numbers are available. Since there is a wide divergence of opinion regarding suitable methods for preservation of complement, it seemed desirable to study some more widely used methods and modifications these suggested.

Shrivindt and Liberman¹ advocated the addition to fresh complement of 10 per cent crystalline sodium chlorate with 4 per cent crystalline boric acid for storage at room temperature, and for storage at 5° C the addition of 10 per cent crystalline sodium acetate with 4 per cent crystalline boric acid, reporting both preserved complement equally well. Sonnenschein² stated that complement was better preserved by using 10 per cent sodium acetate with 4 per cent boric acid than by 10 per cent sodium chloride with 4 per cent boric acid. Midamadi and Giese³ preserved fresh guinea pig serum satisfactorily by adding an equal amount of 12 per cent sodium acetate containing 4 per cent boric acid. Austin⁴ found that a 25 per cent salt solution would preserve complement for two to three weeks when stored in an ordinary test tube at icebox temperature.

Ginsburg and Kalinin⁵ found full complement activity retained for three to four months at room temperature when 10 gm of sodium chloride with 4 gm of boric acid were added to 100 cc of guinea pig complement, using these in dry form rather than in solution as most other investigators had done. The same workers⁶ reported the use of 5 per cent strontium chloride with 4 per cent boric acid was satisfactory. Ruffner⁷ reported that 10 per cent sodium acetate or sodium chloride with 4 per cent boric acid was the best of many preservative substances studied since complement so treated kept four weeks with little change. Complement diluted to 40 per cent with a 12 per cent sodium acetate solution made up in physiologic saline retained its activity for three months at icebox temperature, and for several weeks at room temperature, according to Rhamy.⁸ He pointed out that acetate was not "antibacterial," since growth took place when serum was contaminated, but that it preserved complement in some way by a physicochemical action which involved entering into a loose combination with the

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complement. Ronchese⁹ added 0.04 gm. of sodium fluoride per c.c. of complement, and found that its hemolytic activity remained constant during the first five days; it decreased "two-fold" during the next five days, and "three-fold" during the third five days.

Kolmer¹⁰ investigated sixteen methods for the conservation of complement. He concluded that sodium chloride (0.17 gm. for each c.c. of complement) and storage at a low temperature yielded the best results. He recommended the method of Neill, who added 0.1 c.c. of a saturated sodium chloride solution to each c.c. of fresh complement when dilutions of 1:10 were used, and reported satisfactory preservation for two weeks when treated complement was kept at a low temperature. Kolmer¹¹ advocated the use of 25 per cent sodium chloride when a complement dilution of 1:30 was used. The National Institute of Health¹² reported complement preserved by the addition of 0.1 c.c. of saturated sodium chloride solution to each c.c. of serum maintained a satisfactory titer for six weeks. Complement preserved with 17 per cent sodium chloride with 0.25 per cent chinolol to maintain sterility kept the activity of the complement at its original level for twenty days according to Glover.¹³

Using hydrogen, nitrogen, carbon monoxide, and carbon dioxide to displace air, Valley and McAlpine¹⁴ found only carbon dioxide valuable in preservation, complement titer remaining unchanged for several months. Valley¹⁵ explained this preservative action was due to creation of conditions favoring reduction and preventing oxidation.

Ruediger^{16, 17} used glycerol and freezing for preserving complement and reported Rhamy's method of preserving by sodium acetate a failure. Norton, Barfield, and Falk¹⁸ found that at 37° C. complement completely deteriorated in about three days; whereas at 4° to 6° C. the original titer was maintained for forty-eight hours. When frozen and held at -10° C., it was usable for two weeks. Complement kept at -12° C. retained its hemolytic power for six weeks according to Browning and Mackie.¹⁹ Bigger²⁰ determined the loss of activity of guinea pig serum at 50°, 40°, 30°, 20°, 9°, and 1.5° C. and found more rapid losses occurred as temperature increased.

Dean²¹ failed to preserve complement titer in a dried form after precipitation with alcohol and ether. Flosdorf and Mudd²² reviewed the development of drying as a method for the preservation of complement and described a procedure and apparatus for the preservation of sera, miscellaneous proteins, enzymes, viruses, and bacteria in "lyophile" form. Complement preserved by this method was best stored at 2° to 6° C., and storage for at least ten months did not result in a detectable drop of titer. Eagle, Strauss, and Steiner²³ found complement dehydrated from the frozen state, using the Flosdorf-Mudd apparatus, retained its full hemolytic activity throughout the experiment (eight months) stored at 2° to 6° C. Boerner and Lukens²⁴ used "lyophile" complement prepared from the pooled sera of 10 to 50 guinea pigs and obtained greater uniformity, little waste, and avoided the necessity of obtaining fresh complement during the summer. These same workers later²⁵ reported that "lyophile" complement stored at 8° to 10° C. retained its full hemolytic power for twelve months, some deterioration appearing after a year.

Greaves and Adam²⁰ recently described a procedure for the desiccation of antisera, complement, and bacterial cultures which were frozen by rapid evaporation in a high vacuum in the presence of phosphorus pentoxide. Pooled complement preserved by this process over a four month period maintained a titer comparable to fresh complement.

EXPERIMENTAL

Materials.—Complement was obtained from healthy male or nonbreeding female guinea pigs, all one year old or more. These were housed in an animal house and fed a complete commercial feed which was removed eighteen to twenty-four hours before bleeding. The sera of from 6 to 15 animals were pooled to make up the sample. The sample of Lvo (complement*) was reported to have been prepared from the pooled sera of fifty males.

In obtaining the blood for complement guinea pigs were lightly etherized and 2 cc of heart blood withdrawn. This was placed in a sterile agglutination tube, slanted, allowed to stand at room temperature for one hour and then placed in a refrigerator (7° to 9° C) for twenty-four hours before the titer was determined. Each sample was centrifuged to obtain clear serum.

Corpuscles were obtained from a ewe blood from the jugular vein being aseptically aspirated into a 30 cc syringe and immediately expelled into a sterile flask containing glass beads shaken for about ten minutes to defibrinate the blood, and refrigerated. The corpuscles were washed the same day, three times or more, packed by centrifuging for twenty minutes made up to a 50 per cent suspension, refrigerated and used within two days. The corpuscles were made up to a 2 per cent suspension in saline for use in the tests.

The same antisheep hemolysin was employed throughout the experiment. Frequent titrations showed that no deterioration had taken place. Saline² was prepared by dissolving 8.5 gm C P sodium chloride and 0.1 gm magnesium chloride in cold tap water making up to one liter in volume and autoclaving at fifteen pounds' pressure for thirty minutes.

Various samples of complement in 3 cc portions were stored in sterile rubber stoppered, glass vials with the exception of samples preserved with carbon dioxide, which were plugged with cotton. One cubic centimeter samples were used for freezing experiments. Sera receiving 0.04, 0.05, 0.1, 0.17, or 0.25 gm of a chemical preservative per cubic centimeter were considered as containing 4, 5, 10, 17, or 25 per cent respectively. Time consumed for removal from the refrigerator, sampling, and returning was approximately two minutes for refrigerated samples. All samples of fresh complement were stored at both room and refrigerator temperatures unless otherwise noted. Room temperatures ranged between 21° and 26° C and electric refrigerator temperatures between 7° and 9° C, except when the freezing compartment was used which was approximately -8° C.

Complement Titration.—Complement was titrated by determining the least amount necessary to bring about complete hemolysis of 0.5 cc of 2 per cent sheep

*Printed circular LR 44 & accompanying packaged serum. Mulford Biological Laboratories Sharp & Dohme Philadelphia, Pa.

cells with two units of hemolysin. Complements were returned to isotonicity in diluting them 1:10, 1:20, or 1:30 according to the method of preservation. Unless otherwise noted, a 1:10 dilution was used, the amounts ranging from 0.06 e.c. to 0.30 e.c., with a 0.02 e.c. interval. When other dilutions were necessary 0.1 e.c. to 0.6 e.c. of 1:20 and 0.1 to 0.9 of 1:30 were used, with a 0.05-e.c. interval. The total volume was made up to 3 e.c. with saline. Saline, hemolysin, and complement controls were set up for each titration, and results were read after one hour's incubation in a water-bath at 37° C.

In determining the effect of various methods of preservation on hemolytic titer, 0.30 e.c. of a 1:10 dilution, or its equivalent, was considered as the titer beyond which complement would be valueless in complement fixation reactions. All titration figures given refer to the use of complement in dilutions of 1:10. Tables I, II, III, and IV, contain the results obtained with the various methods of preservation, including the time at which the end points were reached, or at which the samples were depleted or contaminated.

FRESH COMPLEMENT

Controls.—Three samples of complement, in duplicate, were stored without the addition of any preservative. Unpreserved complement held at room temperature had decreased in titer beyond 0.30 e.c. by the third or fifth day, when titers were determined. Less decrease occurred in the refrigerator, a titer beyond 0.30 e.c. not appearing until the tenth or twelfth day, although probably occurring sooner since there was an interval of one week between titrations (Table I).

TABLE I
HEMOLYTIC TITER OF UNPRESERVED COMPLEMENT
(Results Expressed in e.c. of a 1:10 Dilution)

TEST	FRESH	STORAGE				
		PLACE	DURATION—DAYS			
			3	5	10	12
I	0.04	Refrig.	-	0.18	-	0.30+
		Room	-	0.30+	-	-
II	0.06	Refrig.	0.18	-	0.30+	-
		Room	0.30+	-	-	-
III	0.08	Refrig.	0.14	-	0.30+	-
		Room	0.30+	-	-	-

Carbon Dioxide.—Three samples of complement, two in duplicate, were preserved by displacing oxygen in the containers with carbon dioxide. The sample was placed in a cylindrical museum jar, a vaselined rubber gasket applied, and the top, which had two openings, was clamped shut. Carbon dioxide was added through one opening and oxygen was forced out through the other, and after approximately three minutes, when practically all oxygen was displaced, the openings were closed. Two sets were maintained at room and refrigerator temperatures while the third was kept only in the refrigerator. The titers of both samples kept at room temperature had decreased more than half at the end of the first week. Samples held in the refrigerator were superior to those at room temperature since the first sample maintained its original titer for three

weeks, and when depleted at thirteen weeks had dropped only 0.06 c.c. in titer. The other refrigerated samples gave good titers for three weeks and four weeks (samples 3 and 2 respectively.)

Sodium Acetate—A 12 per cent solution of sodium acetate in 0.85 per cent sodium chloride was prepared and sterilized. Duplicate samples were prepared by mixing four parts of complement and six parts of the acetate solution, the mixture containing approximately 8 per cent sodium acetate. A 1:20 dilution was prepared for titration. Our results would indicate that at room temperature this method was not useful as the titer dropped 0.12 c.c. at the end of one week. At refrigerator temperature the original titer was approximately maintained for two weeks, after four weeks there was a difference of only 0.04 c.c., and after nine weeks only 0.07 c.c. Accordingly this method at refrigerator temperatures preserved complement for nine weeks when the original sample was sufficiently active.

Two samples of 10 per cent sodium acetate with boric acid were prepared in duplicate, one by adding 0.1 gm. of sodium acetate and 0.04 gm. of powdered boric acid to each cubic centimeter of serum; the other, by substituting 0.03 gm. of crystalline boric acid for 0.04 gm. of powdered boric acid. This was done because some precipitate was noticeable in the first sample and the crystalline boric acid went into solution much more readily. The first sample at room temperature held its original titer for one week and during the next two weeks the titer dropped only 0.02 c.c. The second sample dropped 0.04 c.c. at the end of one week without further decrease through the fifth week. A reasonable titer was maintained at room temperature for three weeks. Storage at refrigerator temperature gave better results both samples dropping only 0.06 c.c. during six weeks' storage.

Sodium Chloride—A saturated solution of C.P. sodium chloride was sterilized and one sample in duplicate prepared by adding 0.1 c.c. to each c.c. of serum; the mixture containing approximately 3 per cent sodium chloride. At refrigerator temperature the complement held its original titer for two weeks and although the titer more than doubled by the sixth week due to originally high titer the complement was still relatively active. At room temperature, the original titer was maintained for one week more than doubled during the second week and by three weeks reached the selected limit.

Samples prepared with 10 per cent sodium chloride with boric acid were treated as with 10 per cent sodium acetate with boric acid. A considerable difference between samples at room temperature was noted, the first deteriorated rapidly, while the second varied only 0.02 c.c. from its original titer over a period of five weeks. Held at refrigerator temperature, the first sample kept its original titer for two weeks but doubled it during the third week, and at the end of seven weeks a difference of 0.10 c.c. was noted. The second sample showed a difference of 0.02 c.c. for the first six weeks and 0.06 c.c. at the end of eleven weeks. The two samples had the same titers at seven weeks but the actual loss of potency was greater in the first sample, emphasizing that only sera of high titers should be preserved and stored, an observation frequently made during this experiment.

TABLE II

HEMOLYTIC TITER OF COMPLEMENT PRESERVED BY VARIOUS METHODS
(Results Expressed in c.c. of a 1:10 Dilution)

TREATMENT	PER CENT	FRESH	PLACE	STORAGE														
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Carbon dioxide (1)		0.08	Refrig. Room	0.08	0.08	0.08	0.10	0.10	0.10	0.10	0.10	0.12	0.14	0.14	0.14	0.14	0.14	0.14
				0.18	0.18	0.18	0.18	0.18	0.18	0.30+							depleted	
Carbon dioxide (2)		0.08	Refrig. Room	0.14	0.14	0.14	0.16	0.18	0.18	0.18	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
				0.26	0.26	0.30+											depleted	
Carbon dioxide (3)		0.10	Refrig.	0.14	0.14	0.16	0.20	0.20	0.20	0.20	0.20	0.22	0.24	0.24	0.24	0.24	0.30+	
				0.08	0.08	0.10	0.10	0.13	0.13	0.13	0.13	0.13	0.18	0.18	0.18	0.20	0.20	0.30+
Sodium acetate*	12	0.06	Refrig. Room	0.18	0.18	0.20	0.23	0.30+										
				0.06	0.06	0.06	0.10	0.10	0.12	0.16	0.20	0.22	0.22	0.22	0.26	0.30+		
Sodium acetate Boric acid	10 4	0.06	Refrig. Room	0.06	0.08	0.08	0.18	0.18	0.30+									
				0.12	0.12	0.12	0.12	0.14	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.22	0.30+	
Sodium acetate Boric acid	10 3	0.10	Refrig. Room	0.14	0.14	0.14	0.14	0.14	0.16	0.30+								
				0.06	0.06	0.10	0.12	0.12	0.14	0.26	0.26	0.30+						
Sodium chloride	Sat.	0.06	Refrig. Room	0.06	0.14	0.30+												
				0.05	0.10	0.10	0.10	0.10	0.10	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.18	0.23
Sodium chloride*	17	0.04	Refrig. Room	0.05	0.10	0.15	0.20	0.23	0.30+									
				0.05	0.10	0.15	0.20	0.23	0.30+									

*Titrations made in dilutions of 1:20. Results converted to 1:10.

†Titrations made in dilutions of 1:30. Results converted to 1:10.

‡Titer of 0.27 c.c. maintained through nineteenth week, titer of 0.30+ reached during twentieth week.

One sample in duplicate containing *17 per cent sodium chloride* was prepared by dissolving 0.17 gm. of sodium chloride in each cubic centimeter of serum. A dilution of 1:20 was prepared for titration. This preserved complement had approximately its original titer both at room and refrigerator temperature for one week, and by the end of the second week the titers were still equal but 0.06 c.e. poorer. After that, the titer of the sample stored at room temperature was far inferior to that held at refrigerator temperature, which assured preserving action for at least six weeks.

One sample in duplicate containing *25 per cent sodium chloride* was prepared by dissolving 0.25 gm. of sodium chloride in each cubic centimeter of serum. A 1:30 dilution was prepared for titration. At room temperature the titer remained within 0.04 c.e. of the original for the first two weeks. At refrigerator temperature the titer remained approximately the same for two weeks, and was slightly more than double at the end of six weeks. Although doubled, the titer then was of such strength that it could be considered usable, because again this original sample was of high titer.

Sodium Salts (Miscellaneous).—Two samples of complement preserved with *10 per cent sodium chlorate with boric acid* were prepared in duplicate by adding 0.1 gm. of sodium chlorate and 0.04 gm. crystalline boric acid to each cubic centimeter of serum. No preservative action was shown at either room or refrigerator temperature.

One sample in duplicate containing *4 per cent sodium fluoride* was made up by mixing 0.04 gm. of sodium fluoride with each cubic centimeter of serum. This method did not preserve complement activity at room temperature, but when stored in the refrigerator, maintained the original titer for two weeks. At the end of six weeks the titer was nearly double but still usable.

Other Salts.—Two samples of *5 per cent strontium chloride with boric acid* were prepared in duplicate by adding 0.05 gm. of strontium chloride and 0.04 gm. crystalline boric acid to each cubic centimeter of serum. No preservative action on complement was shown at either room or refrigerator temperature.

One sample in duplicate containing *5 per cent magnesium sulfate* was prepared by adding 0.05 gm. magnesium sulfate to each cubic centimeter of serum. Room temperature storage proved of no value, while at refrigerator temperature the original titer was maintained for one week, was half at the end of the second week, and by the fourth week it was over 0.30 c.e.

Freezing.—Six 1 c.e. samples were placed in the freezing compartment of an electric refrigerator at a temperature of approximately -8° C. For each titration a sample was thawed and diluted 1:10. This complement was usable at the end of the first week, but by the end of two weeks the titer had more than doubled. All of the above results are shown in Table II.

DEHYDRATED COMPLEMENT

Dehydration.—Eight 1 c.e. samples were dehydrated for *three hours* by the freezing-dehydration method of Flosdorf and Mudd.²² Four samples were held at room temperature and four in the refrigerator. This complement when restored had a titer of over 0.30 c.e. at the end of the first month stored at room

temperature, and a titer of 0.26 c.c. when stored in the refrigerator, reaching a titer of over 0.30 c.c. at the end of the second month.

One 3 c.c. sample in duplicate dehydrated for *twenty two hours** and stored in the refrigerator had a titer when restored at the end of the first month equal to the original fresh serum, the duplicate likewise giving this titer when examined at the end of four months. The 5 c.c. sample of *Lyo Complement* (Sharp and Dohme) used, the original titer and age of which were unknown, gave a titer of 0.12 c.c. The above samples were restored by the addition of distilled water, and diluted 1:10 for titration.

Dehydration and Salting—One 3 c.c. sample of complement in duplicate was dehydrated twenty two hours first adding 0.15 c.c. and 0.30 c.c. of a 17 per cent salt solution respectively, and stored in the refrigerator. When restored to isotonicity and diluted 1:10, the sample with 0.15 c.c. had a titer at the end of

TABLE III
HEMOLYTIC TITER OF DEHYDRATED COMPLEMENT
(Results Expressed in c.c. of a 1:10 Dilution)

TREATMENT	FRESH	STORAGE				
		PLACE	DURATION—MONTHS			
			1	2	3	4
Dehydrated 3 hours	0.06	Refrig. Room	0.20 0.30+	0.30+		
Dehydrated 22 hours*						
Sample 1	0.10	Refrig	0.10			
Sample 2	0.10	Refrig				0.10
Dehydrated 22 hours†	0.10	Refrig		0.10		
Dehydrated 22 hours‡	0.10	Refrig				0.10
Lyo Complement (restored)	0.12	Refrig	Age and titer of original unknown			

*Unsalted

†Salted with 0.15 c.c. and 0.30 c.c. of 17 per cent sodium chloride respectively before dehydration

the second month equal to the original fresh serum, the duplicate containing 0.30 c.c., likewise giving this titer when examined at the end of four months. The results on all the above dehydrated complements are given in Table III.

Restored Dehydrated Complement—A portion of *Lyo Complement*, restored to its original volume but undiluted, was stored in 1 c.c. amounts at -8°C in the freezing compartment of the refrigerator. When thawed at the end of one week a 1:10 dilution gave a titer more than double that of the original and another examined after three weeks was over 0.30 c.c.

Another portion of *Lyo Complement* was preserved with 10 per cent sodium chloride with boric acid by adding 0.1 gm. sodium chloride and 0.03 gm. of crystalline boric acid to each cubic centimeter of restored serum and refrigerated. When diluted 1:10 at the end of the first week, the titer was 0.08 c.c. lower, and by the end of the third week 0.10 c.c. lower.

*Through the kindness of Dr. Harry Eagle of the Johns Hopkins Hospital four samples of complement (two unsalted, one salted with 0.15 c.c. of 17 per cent sodium chloride solution, and one salted with 0.30 c.c. of 17 per cent sodium chloride solution) were dehydrated for twenty two hours.

A sample of restored unsalted complement was preserved with 17 per cent sodium chloride by adding 0.17 gm. of sodium chloride to each cubic centimeter and stored in the refrigerator. When diluted 1:20 at the end of two weeks, the titer had decreased 0.05 e.c.

Carbon dioxide was used to preserve the remainder of the restored twenty-two-hour dehydrated sample to which 0.15 e.c. of 17 per cent salt solution had been added following the procedure used with fresh complement. This was stored in the electric refrigerator and restored to isotonicity before preparing 1:10 dilutions. The titer decreased one-half at the end of the first week and remained the same for six weeks.

The above results on restored dehydrated complement are shown in Table IV.

TABLE IV

HEMOLYTIC TITER OF RESTORED DEHYDRATED COMPLEMENT, FURTHER PRESERVED BY VARIOUS METHODS

(Results Expressed in e.c. of a 1:10 Dilution)

SOURCE	TREAT- MENT	PER CENT	TITER WHEN RE- STORED	STORAGE										
				PLACE	DURATION—WEEKS									
					1	2	3	4	5	6	7	8	9	10
Lyo Comple- ment	Freezing		0.12	-8° C.	0.26	0.26	0.30+							
Lyo Comple- ment	Sodium chloride	10	0.12	Refrig.	0.20	0.22	0.22	0.24	0.24	0.24	0.24	0.24	0.24	0.30+
	Boric acid	3												
Dehy- drated 22 hr. (salted)	Carbon dioxide		0.10	Refrig.	0.20	0.20	0.20	0.20	0.20	0.20	0.22	0.30+		
Dehy- drated 22 hr. (unsalt- ed)	Sodium chloride	17	0.10	Refrig.	0.15	0.15	0.18	0.20	0.20	0.20	0.20	0.20	0.20	0.24†

*Seventeen per cent sodium chloride was titrated in a dilution of 1:20 and results converted to 1:10.

†Depleted.

DISCUSSION

Results with carbon dioxide do not agree entirely with those of Valley and McAlpine,¹⁴ but it did preserve complement in a relatively active state for at least three weeks at refrigerator temperature. Sonnenschein² found sodium acetate preserved complement better than sodium chloride. Our results indicate that if kept in the refrigerator a reasonable titer was maintained for at least six weeks when preserved with either, one not being superior to the other in one instance, while in another slightly better results were obtained with sodium acetate. At room temperature three of four samples were usable for three weeks. Sodium chlorate was not satisfactory, differing from the results of Shirvindt and Liberman¹ who advocated 10 per cent sodium chlorate with 4 per cent boric acid, but our results did accord with theirs, indicating the adequacy of 10 per cent

sodium acetate with 4 per cent boric acid. Likewise we can agree with Ruffner that 10 per cent sodium acetate or sodium chloride, with 4 per cent boric acid, is among the better methods for preserving complement. Our findings would not indicate that complement kept at room temperature maintained full activity for three to four months when preserved in this manner as reported by Gansburg and Kalinin.⁸ Their samples, however, were stored in darkness, while ours were in a glass cabinet exposed to ordinary light in the room.

Magnesium sulfate had only limited preserving power, and that only at refrigerator temperature. Contrary to Gansburg and Kalinin,⁸ strontium chloride failed to show any preserving action, our results agreeing with Ruffner. Saturated sodium chloride gave results that are nearly in accord with those of the National Institute of Health,¹² and indicate that a reasonably strong complement can be preserved for a six week period if the original titer is high. A satisfactory complement was preserved six weeks by treating with sodium fluoride and refrigerating, results materially better than those reported by Ronchese.⁹

When complement was frozen in the ice compartment of an electric refrigerator, its titer would indicate that it could not be considered usable more than a week. Our results do not agree with those of Browning and Mackie,¹⁰ who used a storage temperature of -12°C and found hemolytic power retained for six weeks. A 12 per cent sodium acetate solution preserved complement at a usable titer for nine weeks when stored in the refrigerator, not equal to that found by Rhamy⁸ but indicating, contrary to Ruediger,¹⁰ that the method is valuable.

When a 1:20 dilution is desired, our results showed that 17 per cent sodium chloride, as reported by Kolmer,⁷ was a useful method, reasonable amount of preserving action existing for at least six weeks at refrigerator temperature. Although approximately half of the activity was lost by the sixth week when preserved with 25 per cent sodium chloride and stored in the refrigerator, the titer was such that it was usable, agreeing with Kolmer.⁷

The activity of complement was preserved little if any when frozen and then dehydrated for only three hours. When unsalted complement was dehydrated twenty-two hours, a titer equal to that of the original sample was maintained for four months at refrigerator temperature, the longest time that any dehydrated complement was stored. Our results on dehydrated complement agree with those of Florsdorf and Mudd,¹¹ Eagle, Strauss, and Steiner¹³ and Bocner and Lukens²² and were superior to those secured with the other methods we used. Samples preserved by salting and dehydration, when restored at the end of two and four months had a titer equal to that of fresh serum, indicating that salting and dehydration was superior to any chemical methods tested.

Whether dehydrated complement after restoration to its original volume could be preserved by some method effective with fresh complement was next determined. If so, such a method might prove valuable for the small laboratory where some complement remained after restoration and immediate needs. Freezing in the ice compartment of a refrigerator was of limited value, similar to results obtained with fresh complement. Ten per cent sodium chloride and

boric acid did not preserve restored complement as well as undehydrated serum, nor did carbon dioxide or 17 per cent sodium chloride, although the latter was slightly superior. The complement could be considered usable after preservation and storage at refrigerator temperature by any of these three methods for at least three weeks, although half the complement activity was lost in all cases.

CONCLUSIONS

Unpreserved complements stored at room temperature were not usable when first titrated after three days, refrigerated samples not dropping as rapidly. Storage in a refrigerator was also much superior to room temperature for all preserved complements.

Strontium chloride and sodium chlorate with boric acid did not prove of any value as a preservative for complement at either room or refrigerator temperatures. Magnesium sulfate had limited preserving power at refrigerator temperature.

At room temperature a satisfactory titer of complement was maintained with 10 per cent sodium acetate with boric acid for three weeks, and with one of two samples of 10 per cent sodium chloride with boric acid. A saturated sodium chloride solution preserved satisfactorily for one week and a 17 or 25 per cent solution preserved for two weeks. At room temperature neither 12 per cent sodium acetate solution nor 4 per cent sodium fluoride preserved satisfactorily.

At refrigerator temperature a satisfactory titer was maintained for not more than nine weeks preserved with a 12 per cent sodium acetate solution, and for six weeks with 10 per cent sodium acetate (or sodium chloride) with boric acid, a saturated sodium chloride solution, 17 and 25 per cent sodium chloride, or 4 per cent sodium fluoride.

When carbon dioxide almost completely replaced the oxygen in a closed container in which complement was stored, rather high hemolytic titer was maintained for at least three weeks at refrigerator temperature, but storage at room temperature was unsatisfactory.

Freezing maintained fresh complement at a usable titer for only one week and failed entirely to preserve restored frozen-dehydrated complement.

Preservation by freezing and dehydration of salted and unsalted complement for twenty-two hours, followed by storage in the refrigerator, gave the best results, maintaining the original titer for four months, the duration of storage. Dehydration for only three hours of unsalted complement was not satisfactory.

For preserving left-over restored frozen-dehydrated complement 17 per cent sodium chloride was most desirable if a dilution of 1:20 was desired. Carbon dioxide or 10 per cent sodium chloride with boric acid was found satisfactory when a 1:10 dilution was preferred.

Most methods for the preservation of complement did not maintain the *original* titers upon storage for any considerable time. Accordingly, fresh complement for preservation should originally have a high titer.

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A UNIVERSAL LABORATORY SHAKER*

J. M. FEDER, M.D., ANDERSON, S. C.

THE appliance here described was designed to meet the needs of the average clinical laboratory for an efficient shaking unit, universal in scope.

Though there are several shaking machines on the market and each performs its particular function in a more or less satisfactory manner, it is quite apparent that none make any attempt to include features applicable to each and every shaking operation.

Increasing inroads on the workers' time by various precipitin reactions for syphilis, vaccines, and allergic antigens all call for some mechanical aid whereby this burden can be somewhat lightened.

With the end in view of making an appliance that would embrace all laboratory operations of this nature, we made an inventory of the various uses to which such an apparatus would possibly be put. This list was headed by the Kline slide precipitin test for syphilis. This most serviceable reaction is coming to the fore and its well deserved popularity impressed me at once with the fact that first of all the appliance must be arranged to handle these slides. The Kline test is performed in many laboratories as an eliminative reaction to safeguard recipients in cases where transfusions of blood are required. Usually these are emergencies. It takes four tedious minutes to turn these slides by hand. We found that the technician could make excellent use of this precious time while a properly adjusted machine agitated the slides, more effectively than this could be done by hand, as it gives accurate, methodical shaking, eliminating the uncertain personal equation.

Next in importance, we considered the Kahn test. Here a vastly different type of motion is required. While in the Kline test, we require a gentle, semi-rotary motion, here we require a vigorous to-and-fro shake with the machine opened wide. Along this same line of thought we included vaccines and allergic antigens. The former needs no comment. All too often we spend many minutes attempting to shake smooth a rough vaccine. In regard to the latter, some if not all laboratories are being called upon, or soon will be called upon, to prepare various extemporaneous allergic extracts from time to time. Most common of these is house dust. It is the opinion of this writer, based upon observation, that while it takes several days to extract a dust with intermittent agitation, the molecular friction obtained by shaking one hour at high speed in the apparatus described will suffice.

Perhaps most important of all is the proper shaking of blood-counting pipettes. Errors in the performance of this little task properly lead those com-

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mitted in most clinical laboratories. Few indeed are the technicians equipped with sufficient perseverance to spend the required three minutes agitating these little tubes by hand. As a consequence many blood counts vary outside of the permissible clinical range. While we freely admit that in the average case, an error of a thousand or so in a white count is possibly of no grave importance, on the other hand to those like ourselves who are engaged in some allergic diagnostic work, this much error would completely nullify the value of our leucopenic index.

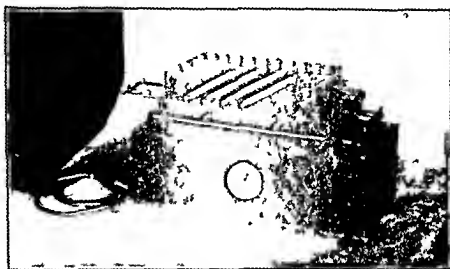


Fig. 1

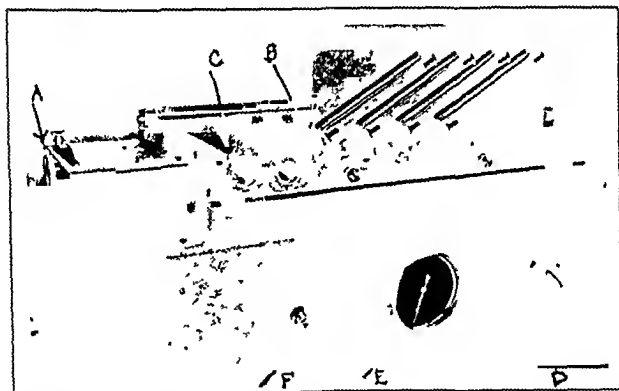


Fig. 2—4. Kline slide holder and nut for its removal. B rack for holding four blood count pipettes. C main shaker table for Kahn racks and miscellaneous articles. D rheostat for speed control. E speed indicator. F snap switch off and on.

In this estimation we pay some attention to a fall of 500 leucocytes and attach definite importance to the fall of 1,000.

It will at once be apparent to all workers that we required a vastly different stroke to properly shake these pipettes than we did to rotate a Kline slide or to rapidly agitate a rack of Kahns or a bottle of vaccine.

Summing up to this point then it will be seen that arrangements would have to be made to take care of at least four types of operation. The slow, gentle

rotation of the Kline slide a rapid, to-and-fro shake for Kahn racks, the adaptation of this same carriage that would hold these so that a small vaccine or other bottle could be accommodated. Finally, the problem of imparting to a rack of blood-count pipettes a combined shaking and rotating motion.

The apparatus finally assembled to perform these tasks is driven by a $\frac{1}{50}$ horse-power motor. Quite obviously there is an optimum speed for each shaking operation, consequently the motor is controlled by a rheostat and number of revolutions per minute noted on a speed indicator. This motive power and control are enclosed in a housing. Mounted on top of this is the shaking unit proper

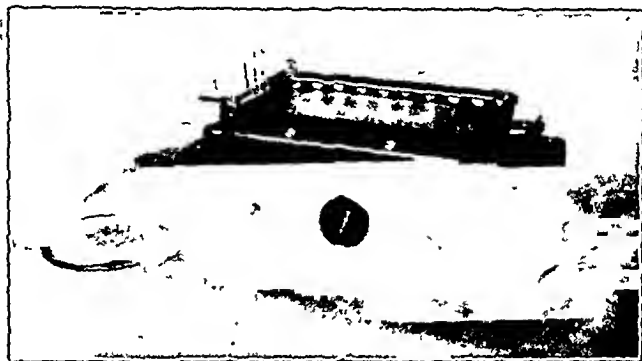


Fig. 3.

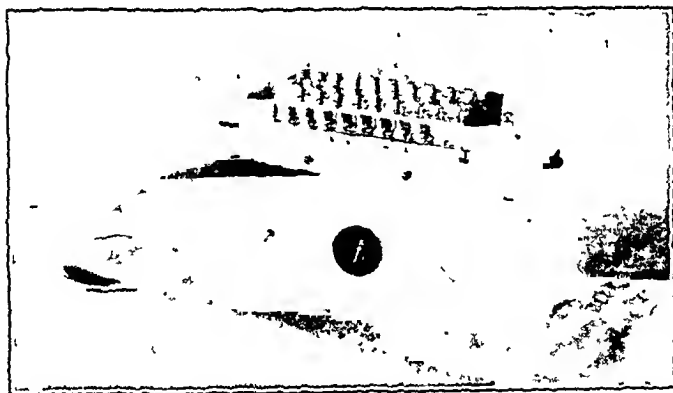


Fig. 4.

as illustrated. Fig. 1 shows the machine with all attachments in place. These can each be readily removed as required. In this picture one Kline slide is noted on left end. Arrangements can be made to place as high as four of these slide holders on housing. Fig. 2 shows close-up with key letters designating various parts. Fig. 3 shows all attachments removed except the sliding bar with which the shaking tray is equipped. This adjustable bar is designed to hold any object from two Kahn racks to one 2 c.c. test tube. It is freely movable and easily locked in place by two knurled nuts. Fig. 4 shows the appliance being used as a Kahn shaker, carrying two fully loaded racks.

The unit is exceedingly well balanced and vibration has been further reduced by mounting the apparatus on four vacuum cup rubber feet. Thus an annoying feature common to most rapidly moving machinery has been practically eliminated.

Optimum speeds have been determined by experiments to be as follows: for the rotation of Kline slides about 125 r p m, for pipette shaking about 225 r p m, and for shaking Kahns, vaccines, etc. 250 r p m.

CONCLUSION

A simple, practical, timesaving universal appliance designed to meet every conceivable clinical laboratory need for an effective shaking apparatus has been discussed.

This apparatus is manufactured and distributed by Phipps and Bird, 916 L. Cary St., Richmond, Va.

article we have had the opportunity to make several observations in connection with the Kline test. We are convinced that to the specificity of this reaction in our hands. This has been so in Kline diagnostic test that we have felt justified in eliminating sion test.

It has been noted that we have departed from the annoying doubtful reaction since by the use of this mechanical rotation the reaction either breaks completely or else smooths out into a perfectly negative test.

We have found that gently mixing the serum and antigen slightly spreading out within the ring using a toothpick has added materially to our results.

Optimum rotation time has been determined to be five minutes and optimum speed at from 100 to 125 r p m.

Gratitude is hereby expressed to Dr. Warren T. Vaughan of Richmond, Va., Dr. Frank Wrenn, Medical Director of this Hospital, and to Mrs. Nan Stewart, my chief technician, for their aid and helpful suggestions in perfecting this instrument. I am also most grateful to the engineering staff of Phipps and Bird for their untiring zeal in assisting with the designing and assembling of the working model.

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A LOW-TEMPERATURE EVAPORATOR*

DANIEL S. STEVENS, PH.D., CHICAGO, ILL.

MANY substances, especially those of biological interest, are sensitive to temperature and so can best be isolated or concentrated with the aid of evaporation at low temperature. The machine shown in Fig. 1 has proved to be a rapid and convenient device for this purpose.

A metal tube (1) about 22 cm. in diameter and 150 cm. long is made into a vacuum tight chamber by soldering a cap (2) on one end and fitting a gasket-sealed door (3) on the other end. A plate (4) carries the dishes which hold the material to be evaporated. In our case these are five standard Pyrex trays 26 cm. by 16 cm. by 5 cm. Ten flat iron heating units are fastened to the under side of the plate (two in series under each dish).

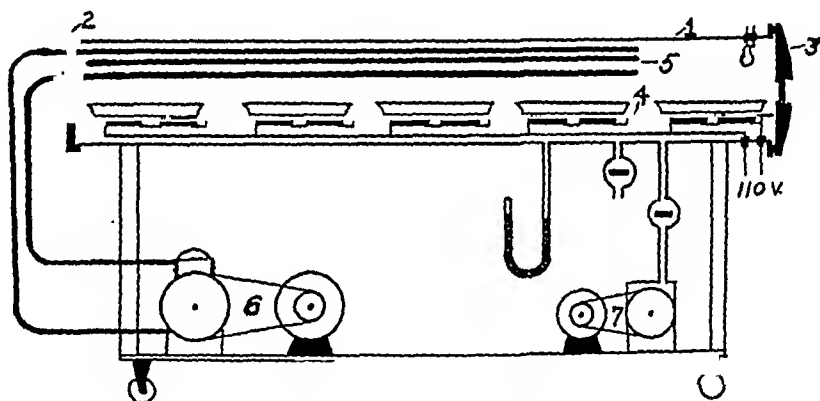


Fig. 1.—Low-temperature evaporator for laboratory use.

A refrigerating coil (5) occupies the top of the cylinder. This is made of three loops of 2 cm. copper tubing, having a length of 120 cm., which is 30 cm. shorter than the chamber, to leave space for tall flasks. The coil can be cooled to about -20° C. by the $\frac{3}{4}$ H.P. refrigerating unit (6). The chamber is evacuated by an oil pump (7).

To charge the machine, about 500 c.c. of solution is placed in each dish. The refrigeration unit, which is automatic in operation, and the vacuum pump are turned on. The interior is illuminated by an electric light, so that the dishes can be observed through a window in door (3). When the pressure in the chamber has been reduced to the vapor pressure of the solution, the liquid boils. The pump is then stopped and need not be operated again. The current through the heating elements is set at a value that will maintain the desired temperature in the dishes.

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The vapor from the solution condenses on the refrigeration coil. After the desired amount of evaporation has occurred, the dishes are removed and a long metal tray put in their place. The refrigeration unit is stopped and the coil defrosts into the tray.

If it is desired to evaporate the solution from the frozen state, the pumping is continued intermittently until it has turned to a solid. A thermoelement fastened to the side of a dish can work a relay in the heating circuit. A satisfactory element is a mercury thermometer with electrical contacts sealed into it. These can be obtained for operating at different temperatures, and standard relays have been designed for use with them. The thermoregulator is especially valuable when evaporation of the frozen material is required.

The machine described will evaporate in twenty-four hours about 2,500 cc of water at 8° C. About half this quantity of ice can be evaporated in the same time. The rate of evaporation rises rapidly with increased temperature and can also be increased by defrosting the coil more than once a day.

Vapors such as benzene and water will freeze on the coil without difficulty. Sometimes other volatile constituents are present. Ammonia gas will freeze with

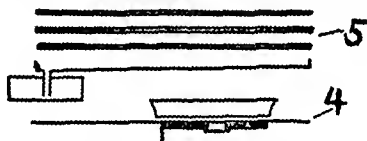


Fig. 2—Arrangement for evaporating low freezing liquids

the ice without trouble. The vapor from an alcoholic solution may have a freezing point lower than the temperature of the refrigeration coil, especially since the vapor is much richer in alcohol than the original solution. In this case, liquid condenses on the coil and drips back into the dish.

However, alcoholic solutions can be handled by using the arrangement shown in Fig. 2. A tray with a reservoir is placed between the dish and the coil to catch the liquid that drips down. The presence of about 5 per cent alcohol in the solution is desirable for easy defrosting. This concentration can be frozen on the coil and the low melting layer next to the coil causes the ice to break away readily upon defrosting.

This machine is one of moderate size for laboratory use. A few machines have already been built of varying size. Their use on various products has shown that this device is not only convenient but may be necessary to prevent undesirable changes in sensitive materials. The product obtained from the evaporation of ice is very finely divided. Solutions, either liquid or frozen can be evaporated from the inside of ampoules.

The author is indebted to Dr. George F. Dick and Dr. Gladys H. Dick for suggesting the construction of this machine and the Scarlet Fever Committee for its financial support.

COMBINATION MICROHEMOPIPETTE*

FOR DETERMINATION OF THE SEDIMENTATION RATE, PACKED CELL VOLUME, AND
FRAGILITY OF ERYTHROCYTES, ESPECIALLY ADAPTED FOR USE IN CHILDREN
AND SMALL LABORATORY ANIMALS

KATSUJI KATO, M.D., PH.D., CHICAGO, ILL.

IN BOTH clinical and experimental studies of the blood such procedures as the estimation of the sedimentation rate, of the packed cell volume, and of the resistance of erythrocytes against various hemolytic substances, are increasingly being employed as a means of obtaining necessary information for accurate diagnosis and prognosis of anemic states. For the routine red cell count the use of standard blood diluting pipettes and counting chamber is now so well established that further improvement seems unnecessary. For the determination of the hemoglobin content of the blood several excellent methods are widely used with entire satisfaction, particularly those based on colorimetric principle of acid hematin (Newcomer, Haden-Hausser, Sahli, Hellige and Hellige-Wintrobe). But for the less frequently employed tests the instruments as well as the methods are so diverse and some even so cumbersome that great difficulty is experienced in selecting a procedure suitable for all purposes. A perusal of the rapidly growing literature dealing with the numerous devices and techniques for hematocrit, sedimentation, and fragility tests naturally suggests a need for the simplification and standardization of the instrument, incorporating the essential features inherent in each existing method. A combination or all-purpose microhemopipette here described has been devised in an attempt to fulfill this particular need. A serious attempt has been made to meet the four fundamental requirements which all improved laboratory methods should possess: (1) simplicity in both the instrument and in its manipulation, (2) maximum accuracy, (3) universal applicability, and (4) manifold usefulness.

In a comparatively brief period of time during which this new pipette has been used for various tests, I have found it to be satisfactory and timesaving, especially suitable in pediatric practice. The present report is merely a description of the instrument and the various methods of its use; the actual results of investigation, together with normal standards for each of the tests for which this pipette can be used, will be reported in subsequent publications.

DESCRIPTION OF THE PIPETTE

The pipette is a straight capillary tube of about a 5 mm. outside diameter, with a uniform bore of slightly less than 1 mm. diameter, and 12 cm. in length

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(Fig 1A) The proximal end of the pipette tapers to a diameter of approximately 4 mm so as to facilitate the attachment of the ordinary rubber suction tubing used for blood pipettes. The distal tip of the pipette is ground down to a diameter of approximately 2.5 mm. As measured from the distal tip, the portion actually used for observation is from 9 to 10 cm in height, and is graduated into 100 equal parts of about 1 mm each. The content of the bore so graduated is calibrated to hold exactly 50 cmm of blood. Since the accuracy of this volumetric calibration is given first consideration in the manufacture of the pipette, the actual height of the used portion of the bore varies inversely

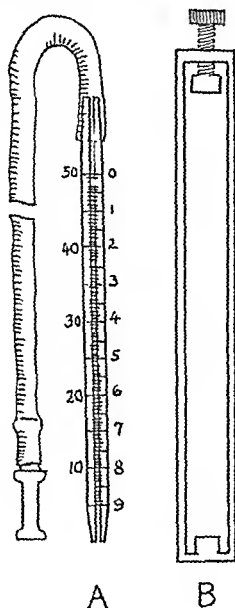


Fig 1—Combination microhemopipette (Kato) 1 Pipette B, metal frame (Two thirds actual size)

according to the diameter of the bore in a given pipette. Thus by actual calculation it is found that only when the diameter of the bore is 0.79 mm does the height of the used portion of the pipette approach 10 cm for 50 cmm of volume content. Since absolute adherence to this particular ratio would be relatively costly in manufacturing the instrument and inasmuch as the small amount of deviation from this ideal proportion is not detrimental to the macroscopic interpretation of the results a tolerance of 1 cm in height is permissible without sacrificing the essential accuracy of the pipette. Two series of graduation numbers are etched on either side of the linear markings of the pipette, one series

on the left side (10, 20, 30, 40, and 50, reading upward) designating the volume content of the bore in cubic millimeters, and the other on the right side (0, 1, 2, 3, 4, 5, 6, 7, 8, and 9, reading downward) indicating the percentage figures when one eipher is added.

For centrifugation of the pipette a special metal frame has been evolved (Fig. 1B). This frame is constructed of a narrow metal sheet (brass or stainless steel), $\frac{1}{16}$ inch thick and $\frac{3}{8}$ inch wide, welded together at one end into a narrow rectangular shape, the total length being about 13 cm. and the width about 1 cm. The clearance space is thus just sufficient to accommodate the pipette. The inner surface of the bottom of the frame is provided with a small metal cup which receives the distal end of the pipette; the proximal end of the pipette is im-

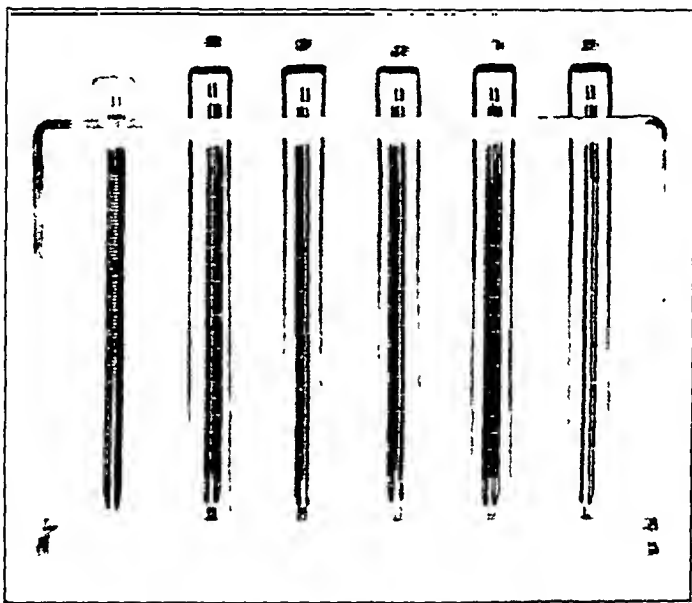


Fig. 2.—A set of six combination hemopipettes in metal frames placed in vertical position on a specially devised rack.

mobilized by a similar metal cup at the top of the frame, which is made adjustable by means of a small screw, approximately 2 cm. long, manipulated by a thumb piece outside the frame. Both the upper and lower metal cups are cushioned with small circular pieces of hard rubber to protect the tips of the pipette against breakage. The pipette is always inserted into the frame in a horizontal position, since the content of the pipette may be easily lost in vertical position. After the pipette is securely held in position, the frame and the pipette are set up in a suitable rack at vertical position (Fig. 2). For the sedimentation rate readings the fall of the erythrocyte column is noted at desired intervals; it is then placed in a 6 inch centrifuge cup and the packed cell volume obtained after fifteen to twenty minutes of centrifugation at 2,000 to 3,000 revolutions per minute. A special technique is required for the fragility test of erythrocytes, which will be reported later.

METHOD OF OBTAINING BLOOD SAMPLES

Capillary blood is recommended for performing various tests with the combination pipette, although venous blood whenever available, can also be used with equal satisfaction. Venepuncture in infants and young children, or cardiac puncture in small experimental animals (rats, guinea pigs, and rabbits), is less convenient than pricking the finger or ear lobe. The object of the micropipette method is to enable the worker to perform various tests from the same small amount of blood readily obtainable by capillary puncture. From 0.2 to 0.3 cc of blood is sufficient for performing all necessary tests, including sedimentation rate, packed cell volume, and hemoglobin determination, as well as red and white cell counts. With this amount of blood even the fragility tests can be performed, providing every drop can be utilized without waste. Skill and speed in manipulation, acquired after a little practice will enable the worker to perform a surprising number of tests by the use of this pipette using as small an amount of blood as is obtainable from a capillary puncture.

As a receptacle for the blood sample a hanging drop slide with a central depression (15 mm in diameter and 3 mm in depth) is the most convenient. The surface of the depression is previously coated with a 2 per cent solution of oxalate mixture and dried. The oxalate mixture may be prepared by dissolving 0.8 gm of potassium oxalate and 1.2 gm of ammonium oxalate in 100 cc of distilled water (Heller and Paul¹). The drops of blood from a puncture wound are received into the oxalate coated cup of the slide and quickly agitated by means of a fine glass rod. The depression of a hanging drop slide of the above mentioned dimensions holds, when filled to the brim, approximately 0.5 cc of blood. The worker must make sure that no coagulation has taken place before removing the sample to the laboratory for study. In a dry or warm season or climate the slide should be placed at once in a moist chamber (such as a Petri dish with a moist filter paper placed at the bottom) to avoid evaporation of the blood.

OTHER USES OF THE INSTRUMENT

Since the bore of the combination hemopipette is accurately calibrated it can be used for obtaining precisely measured amounts of blood for various quantitative tests for which micromethods are available. The pipette is especially useful for removing samples of blood for use in the acid hematin method hemoglobinometers. Thus for the Newcomer apparatus, exactly 10 cmm of blood is transferred into a test tube containing exactly 5 cc of 1 per cent hydrochloric acid. Similarly for the Sahli apparatus, exactly 20 cmm of blood is placed in a Sahli tube containing a measured amount of a N/10 hydrochloric acid. For other chemical analyses of the blood, particularly estimation of blood cholesterol, glucose, and minerals, the combination hemopipette offers a convenient means of obtaining accurately measured quantities of the blood.

In serodiagnosis of syphilis an increasing number of micromethods are being devised (micro Kahn² and capillary Hinton³ tests), and the technique of mixing the serum and the antigen in specified amounts will be greatly facilitated by the use of the combination pipette. More recently Isaacs⁴ devised a cell count method

using bone marrow obtained at either autopsy or biopsy, and the technique of mixing the marrow material and the diluting fluids can be accomplished with relative ease by use of the combination pipette.

ADVANTAGES OF COMBINATION HEMOPIPETTE

The combination microhemopipette here briefly described is recommended to clinical and laboratory workers for more extensive trial and use on the basis of its distinctive advantages, summarized as follows:

(1) Being a capillary pipette, it requires only a small quantity of blood such as is obtainable by the routine skin-puncture method, without recourse to venepuncture in human beings or to cardiac puncture in small animals. This is an important practical consideration, particularly appreciated by pediatricians and by experimenters using small laboratory animals.

(2) The combination pipette with its metal frame, itself simple in construction, requires no additional laboratory equipment other than the ordinary type of centrifuge capable of a speed of 2,000 to 3,000 r.p.m. If for any reason the centrifuge at hand cannot develop a speed exceeding 2,000 r.p.m., a longer time must be allowed for complete packing of the red cells.

(3) The manifold usefulness of the combination pipette is attested by the fact that it can be utilized for the determination of the packed cell volume, of the suspension stability of erythrocytes, and of the fragility of red blood corpuscles against hemolytic substances. The pipette can also be used for removing precisely measured amounts of blood for various quantitative studies, particularly hemoglobin estimation by the acid-hematin method. It may also be used to advantage in mixing the serum and antigens in specified amounts in carrying out the microprecipitation tests in the diagnosis of syphilis.

(4) The universal applicability of the combination pipette to all types of hematologic examination in all age groups, but particularly in infants and small animals, facilitates performing the cell volume, sedimentation and fragility tests more frequently than is convenient with the more elaborate and cumbersome techniques, which in turn materially contributes to a more accurate diagnosis and prognosis of anemias. It is suggested that the combination microhemopipette be made a part of the regular hematologic equipment for routine procedures.

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USE OF DAPHNIA IN STUDY OF CATHARTIC ACTION*

WILLIAM TINSLEY (CHICAGO, ILL.)

THE use of daphnia as an experimental animal is not new as its availability, inexpensiveness, and ease in culturing make it an attractive form for study. Daphnids have been used widely in Russia¹ and some work has also been done in England with them² and in Canada³ mainly in the purely biologic field. Its reactions have been studied by these observers under the influence of various nutritive media, light, and heat.

Aino Viehovei⁴ has to our knowledge been the first to use daphniae as a means of bio assay. His published work on *Aloe Evaluation*⁵ suggested the use of daphniae for the study and evaluation of cathartics and his method was tried at first, but we found it necessary to modify it to a certain degree to obtain more consistent results in our hands.

Daphnia magna, the preferred species for this work is a minute crustacean belonging to the family Daphnidae tribe 2 or Anomopoda (characterized by 5 or 6 pairs of trunk limbs, the first two pairs more or less prehensile) of suborder 1, the Calyptomeira (carapace completely enclosing body and limbs), which belongs to the Cladocera suborder 4 of the crustacean subclass Branchiopoda⁶.

CULTURING OF DAPHNIAE

After a study of the various types of culture media suggested by Banta⁷ and Viehovei,⁴ we have come to use the infusion prepared with dried sheep manure (Whizard brand), as suggested by Viehovei. It is to be understood at the outset that different batches of the culture medium will vary to a greater or less degree in constituents but that if it is prepared in the same way each time, any error is compensated for in the course of a sufficient number of observations.

Preparation of Culture Medium—Whizard brand dried sheep manure is used in the proportion of 1 gm. to every 1,000 cc. of tap water. A large container, preferably of earthen ware is filled with chlorine free tap water, and the required amount of the dried manure is placed in a small gauze bag and suspended in the water. After twenty-four hours the bag is removed. The water has usually turned a deep brown color at this time, but only a small number of infusoria and bacteria is present. The fluid is allowed to stand another twenty-four hours at room temperature and at the end of this time a microscopic examination is made for infusorial forms which not only serve as food for the daphnia, but also indicate that the medium is suitable for the development of minute animal forms. The growth should be quite abundant by this time. If it is absent and does not show up by the end of another day, the contents of the container are discarded and the container itself is thoroughly scrubbed, as the infusoria

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of infusorial growth after seventy-two hours is usually due to some contamination of the creek or water, sufficient to render the medium unsuitable for culturing of daphniae. We prepare and use for stock cultures fresh culture medium weekly, as the fauna and flora change on prolonged standing.

Placing the manure in a gauze bag eliminates the necessity of straining the liquid to remove excess organic matter that might cause decomposition of the medium. The resulting almost clear fluid is of light brown color and contains an abundant supply of fodder organisms necessary to maintain a healthy well-filled gut in the daphnia. When the daphniae are first placed in this medium, or when they are changed from one culture medium to the other, the gut may become emptied, and it may require a period of time to refill. For this reason daphniae which are once started in a sample of culture medium should be left in that sample, and water from *that* sample be used in the particular experiment with the daphniae taken from it. The effect on the metabolic rate of the daphniae resulting from changes in the culture medium due to aging of the medium and also due to changing to water of different sources or temperature, has been reported upon in an article by Warren.³

Because of the susceptibility of the daphniae to chlorine, it is necessary, as previously indicated, that the tap water used be free from chlorination.

It is advisable to check on the hydrogen ion concentration of the medium. Viehoever suggests the addition of an excess of calcium carbonate to the culture water to aid in maintaining an even pH.⁴ In the course of experiments on the effect of pH changes on daphniae, we found that the culture medium itself has a tendency to maintain a pH of about 8.0. For more complete information on the effect of hydrogen ion concentration on daphniae, one might consult Klugh's work with buffer solutions of dibasic sodium phosphate and sodium acetate.⁵

Selection of Test Daphniae.—Mature daphniae are placed in large culture jars containing the medium.⁶ Care must be exercised not to put an excessive number of daphniae in a given quantity of fluid. Crowding upsets the metabolic rate, probably due to insufficient oxygenation, resulting in lowered heart rate, increased susceptibility to unfavorable influences, shortened tail spine, and the production of males and of winter eggs. Crowding does cut down the number of young produced and daphniae from overcrowded cultures are unsatisfactory for assay work. It has been suggested that 40 c.c. of medium be allowed for each daphnia. We have found that a smaller quantity, but not below 10 c.c. per animal, can be used. Aeration is of advantage. This is done as follows: the jars containing the mature females are placed in a diffuse light (north window) away from fluctuations of the temperature above or below normal room temperature (68° F.). A capillary pipette is placed in each jar and connected with an air line. This is so regulated that a small bubble escapes every one or two seconds, and this is continued for hours at a time. These bubbles in traveling to the surface maintain a slow circulation in the medium and keep it sufficiently supplied with oxygen. The evaporated water should be replenished with distilled water from day to day. If the latter is added in small quantities, it has no deleterious effect on the daphnia.

⁶The culture jars we have found satisfactory measure 6 inches in diameter by 9 inches in height, containing about 4 liters.

Breeding of Test Specimens—A mature gravid female (Fig 1, recognized by the eggs within it) is placed in a liberal supply of culture medium, and multiplication is permitted to take place until a large stock of offspring has developed, which usually requires about two months. The gravid females are then pipetted into other jars, and these brood jars are inspected daily for newly born daphniae. As the daphniae retain the young in the brood sac until they are liberated as free swimming forms, it is a simple matter to pipette out the crop of young each day. The young daphnia is visible to the naked eye when born. The day's crop of young may be counted as they are removed and placed into other jars, these jars are then marked with the birth date and number of young and set aside. Such fleas as are not used experimentally are added to the stock

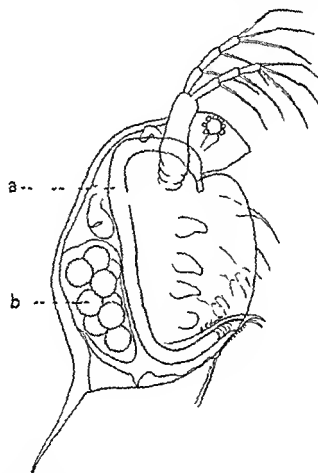


Fig 1—Mature gravid female daphnia a Gut b Ovary

jars of gravid females to supply future cultures. The culture water in these brood jars is changed every other week.

All this preparatory procedure is important because only daphniae of a certain age are suitable for quantitative results.

McArthur and Bailhe⁶ have determined the heart rate to be highest and consequently the metabolic rate to be greatest on the seventh day. In experiments on the effect of age on consistency of results, we have found that this age gives the most consistent data and that the results become more scattered with increasing maturity. As the daphniae become older the carapace becomes thicker and less transparent rendering observation less easy. With maturity and the appearance of eggs in the brood sac, the results are still less reliable, due probably to changes in metabolism attendant upon the appearance of the eggs. Though seven day daphniae would probably be the best for the purpose, we find their small size rather inconvenient. Daphniae ten days of age are large enough

For the subdivision of the gut the following landmarks are employed: 0 per cent at the anterior portion of the tract, 25 per cent at the posterior end of the cardiac flexure, 50 per cent at the midpoint of the tract, and 75 per cent at the beginning of the curve of the postabdomen (Fig. 3a). To facilitate the recording of the amount of material in the gut, the condition of the tract may be noted as "very loose," "loose," and "solid" (Fig. 3b). When the contents present an unbroken, opaque appearance, it is termed "solid." When the tract becomes lighter in color and broken spaces begin to occur, it is called "loose." The appearance of small clumps of material separated by larger spaces indicates a "very loose" condition. When the contents no longer appear in the extreme posterior of the tract, it may be termed empty, even though small particles of food just ingested appear in the extreme anterior end. Recording the consistency of the contents in this manner enables the worker to obtain a fair estimate of the condition of peristaltic activity. An emptying of the tract which presents a solid mass of material moving through the intestine, indicates an increased rate in stool passage. If the solid material begins to loosen at the anterior end and show a tendency to continue loosening, it is almost certain that reverse peristalsis has set in. For quantitative comparison the time of 75 per cent evacuation is probably sufficient.

For work with the sparingly soluble bodies, such as phenolphthaleins, the saturated solution with excess has been used. Present indications are that in the case of more active agents, such as yellow phenolphthalein, the stimulation of peristalsis may actually be so strong as to inhibit evacuation. For this reason it may be necessary to work with dilutions and comparisons made of relative efficiency according to the dilution.

SUMMARY

A method of procedure for the use of *Daphnia magna* in the assay of cathartics is herewith submitted. In order to secure comparable results it is important that a standard technique be established. We believe that the procedure described gives comparable results, although we are willing to admit the possibility and desirability of further improvement and refinement.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D. ABSTRACT EDITOR

TUBERCULIN REACTION, Daily Variations in, Howe J S *Am Rev Tuberc* 37 264, 1938

I Range of Variation in Tuberculous and Control Subjects

II Relation to the Vascular Pressor Episode

There is a definite correlation between the quantitative variations in the tuberculin reaction and the state of the peripheral vessels as measured by daily diastolic blood pressure levels. In this series of six subjects the correlation ranges from 77 per cent to 100 per cent.

Periods of peripheral vasoconstriction characterized by increasing diastolic blood pressure, are accompanied by a decreased reaction to tuberculin and in several instances by completely negative reactions to the smaller dose of tuberculin.

Periods of peripheral vasodilation characterized by a falling diastolic blood pressure following a definite pressor peak, are accompanied by increased reaction to tuberculin. This exaggeration of the tuberculin reaction is most marked immediately following a marked pressor episode, and may subside with complete vasodilation.

In all subjects studied over the same period the trend of the tuberculin reaction at the same time is roughly parallel that is, the peaks and troughs of the curves for the different subjects coincide more or less closely. This is in accord with the work of Petersen who has shown that vasoconstriction and vasodilation reflected in the blood pressure readings are largely influenced by the meteorologic environment particularly by the infall of polar air, which causes peripheral vasoconstriction and is reflected as the 'pressor episode'.

The mechanism of these variations in the tuberculin reaction appears to be one of periodic changes in vascular and tissue permeability affecting the extent of local exudation in response to a definite dose of tuberculin. The increased permeability may also result in an actually increased sensitivity of the cells to tuberculin.

The vascular pressor episode may be reflected not alone in variations in the tuberculin reaction but also in clinical symptoms and chemical and serologic changes.

PHENOLPHTHALEIN STUDIES—Elimination of Phenolphthalein Fantus, B. and Dyniewicz, J M *J A M A* 110 795 1938

Method of Determining Conjugated Phenolphthalein in Urine—Heat 10 cc of urine and 10 cc of 10 per cent hydrochloric acid on a water bath for from three to four hours to break up the conjugation. Allow the solution to cool. Extract with ether in from 15 to 20 cc portions until a small volume of the last washing shows no trace of phenolphthalein when tested with sodium hydroxide volumetric solution.

The combined, filtered ether portions containing the freed phenolphthalein are shaken with a small portion of tenth normal sodium hydroxide volumetric solution and allowed to stand until the mixture has completely separated. The alkaline solution is then drawn off into a second separator, and the process repeated until all the phenolphthalein is extracted. The combined alkaline solutions are then made acid with the smallest amount of hydrochloric acid and again extracted with ether.

The ether portions are made alkaline with tenth normal sodium hydroxide volumetric solution (the volume used depending on the amount of phenolphthalein present) and compared with the same volume of tenth normal sodium hydroxide volumetric solution, to which is added a known standard phenolphthalein solution to color equality, and the amount of phenolphthalein present is calculated.

Method of Determining Free Phenolphthalein in Urine.—Extract 10 c.c. of urine with ether in from 15 to 20 c.c. portions until a small volume of the last washing shows no trace of phenolphthalein when tested with tenth normal sodium hydroxide volumetric solution. The combined, filtered ether portions containing the phenolphthalein are shaken with tenth normal sodium hydroxide volumetric solution, the volume used depending on the amount of phenolphthalein present. It is advisable to make the extraction with as few separations as possible and to make the reading quickly because phenolphthalein in alkaline solution tends to decolorize. This solution is compared with the same volume of tenth normal sodium hydroxide volumetric solution, to which is added a known standard phenolphthalein solution to color equality, and the amount calculated.

INTOXICATION, ALCOHOLIC, Diagnosis and Medicolegal Implications, Selesnick, S. J. A. M. A. 110: 775, 1938.

It is important to have definite criteria as a basis for the diagnosis of alcoholic intoxication in accidents involving individuals who have imbibed alcoholic beverages. The chemical determination of body fluid alcohol offers a scientific means of establishing whether or not an individual has imbibed alcohol and of estimating the degree of alcoholic intoxication. Blood as a medium for analysis is preferable to spinal fluid, urine, saliva, and expired air, because it contains a negligibly small amount of nonalcoholic oxidizable material, its alcoholic content represents the degree of alcohol saturation at the moment the blood sample is obtained, it is always available, and its extraction does not necessitate the active participation of the subject. There are sufficient scientific data to prove that subclinical intoxication—or alcoholic intoxication in the biologic case without any gross manifestations of drunkenness—can produce sufficient interference with psychomotor activity and neuromuscular coordination to render such an affected individual a potential public menace. Blood alcohol determinations can detect these degrees of alcoholic intoxication which ordinarily escape the detection of competent physicians. Criteria, therefore, must be established which include body fluid alcohol determinations as part of the diagnostic armamentarium.

SULFANILAMIDE: Para-aminobenzenesulfonamide, Absorption and Excretion, Method of Determination in Urine and Blood, Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C.: J. A. M. A. 108: 953, 1937.

The authors present the following methods for the determination of sulfanilamide in the urine and blood. The method depends upon the presence of an amino group substituted in the benzol ring. Diazotization with nitrous acid in an acid solution produces a diazo compound with dimethyl- α -naphthylamine, a purplish-red azo dye estimated by colorimetry. The method has a sensitivity of 1 part to 20 million of water.

Reagents:

1. 0.1 per cent freshly prepared solution of sodium nitrite.
2. N/10 hydrochloric acid.
3. 95 per cent ethyl alcohol.
4. Dimethyl- α -naphthylamine 1 c.c. to 100 c.c. of alcohol.
5. Standard solution: 200 mg. of sulfanilamide per liter. From this stock solution standards are prepared containing 1.0, 0.5, and 0.2 mg. per cent. The standards are stable for several months if kept in the icebox.

Method for Urine:

1. Dilute the urine to contain 0.5 to 1.5 mg. per cent of sulfanilamide (with the amount ordinarily administered dilutions of 1:50, 1:100, or 1:200 suffice).
2. To 10 c.c. of diluted urine in a small flask, add 2 c.c. of HCl, 1 c.c. of sodium nitrite, 5 c.c. of alcohol, and 1 c.c. of naphthylamine. Shake the flask after the addition of each reagent.
3. Treat similarly 10 c.c. of the standard solution in a similar flask.
4. Allow both flasks to stand ten to fifteen minutes and match the solutions in a colorimeter.

Method for Blood

1 While shaking, add 1 volume of blood to 9 volumes of alcohol, stopper the flask, allow to stand ten to fifteen minutes (or longer) and filter.

2 Transfer 10 cc of filtrate to a small flask and add 5 cc of water, 2 cc of HCl, and 1 cc of sodium nitrite

3 Allow to stand three minutes and add 1 cc of naphthylamine

4 In a similar flask add 1 cc of standard to 9 cc of alcohol and treat similarly
Allow to stand ten to fifteen minutes, filter, and compare colorimetrically

These determinations give the concentration of free sulfanilamide—not the conjugated form which, because of blocking of the amino group, does not give the color reaction until hydrolyzed by hydrochloric acid and heat

SULFANILAMIDE, Estimation in Blood and Other Body Fluids, Proom, H *Lancet* 234 260, 1938

Reagents

1 20 per cent aqueous trichloroacetic acid

2 0.5 per cent sodium nitrite solution

3 1 per cent alcoholic solution of dimethyl alpha naphthylamine

Method (Applicable to blood, urine, milk, and spinal fluid)

1 Dilute 1 cc of blood (or body fluid) with 2 cc of distilled water

2 Add 1 cc of trichloroacetic acid solution mix and filter through a small paper

3 To 1 cc of filtrate add 0.05 cc (1 drop) of sodium nitrite solution

4 Allow to stand three minutes and add 0.5 cc of naphthylamine solution and mix by gentle shaking

5 Allow to stand fifteen minutes and compare colorimetrically with standard solutions similarly treated

Standards

Prepared either from solutions of phenol red in phosphate buffer or, for concentrations less than 0.01 mg per cc from solutions of cobalt sulfate in dilute sulfuric acid. Standards are sealed in $\frac{1}{2}$ by 4 inch test tubes of nonalkaline glass. Suitable ranges: 0.1, 0.05, 0.025, 0.01, 0.005, 0.0025, and 0.001 mg per cc

Method accurate to within 10 per cent

TISSUE Gum Dammar for Mounting Sections, Evans, N *Arch Path* 25 83 1938

Dissolve the resin in the 'neutral histological (practical) xylene' specially prepared by the Eastman Kodak Company. This is done by placing the ingredients together in a large stoppered bottle in an incubator and stirring with a glass rod several times daily until the mixture is of a heavy syrupy consistency. This is usually accomplished in about a week's time. Then strain through four layers of clean gauze and store in a dark bottle. If the resultant solution is found to be too thin for mounting purposes, a thicker consistency can be secured by placing the unstoppered bottle in an incubator for evaporation until it is of the right consistency. A satisfactory solution is considerably thinner and much less sticky than the preparations of Canada balsam generally used, and can be applied to the slide quickly.

The following advantages are noted for this method:

Slides mounted with gum dammar are much cleaner, dry quicker, will not stick together if allowed to dry for a few months before storing in contact with other slides, do not discolor, and do not fade.

An important advantage is the possibility of storing slides in compact arrangements, since they have no tendency to stick to one another, thus saving much space and expense in providing cabinets for the permanent filing of the slides.

Prepared solutions of gum dammar ready for use can be purchased from laboratory supply houses.

TUBERCULOSIS: The Weltmann Serocoagulation Reaction, Levinson, S. A., and Klein, R. I. *Am. Rev. Tuberc.* 37: 200, 1938.

The Weltmann serum coagulation test is of importance in distinguishing between exudative and productive changes. The technique is simple, and the method is described. In general, acute inflammatory and exudative conditions give a shortened coagulation band or a shift to the left. In chronic diseases, characterized by fibrotic changes, in the healing stage of acute infection, and in parenchymal liver damage, the coagulation band tends to be lengthened, or results in a shift to the right. The coagulation band does not always parallel the sedimentation rate. In tuberculosis the Weltmann coagulation test is of importance because it can be used as a guide in the course of and in the prognosis of the disease. It reflects tissue changes that occur in the body. Exudative changes in tuberculosis are manifested by a shortening of the coagulation band, while fibrotic changes are accompanied by a prolonged coagulation band. When exudative and fibrotic changes occur in the lung at the same time, as happens frequently in tuberculosis, the Weltmann reaction may not be of great assistance. The two divergent processes occurring at the same time result in the coagulation band striking the normal zone. In miliary tuberculosis, peritoneal tuberculosis, and in meningeal tuberculosis, the Weltmann reaction gives a shortening of the coagulation band in the exudative zone. Isolated bone tuberculosis is accompanied by a normal or prolonged coagulation band and is reflected in the fibrotic zone. There is a correlation between the blood pH and the Weltmann reaction, but the authors believe that both of these changes may be directly related to the underlying process. They have applied the Weltmann test not only to tuberculosis in the minimal, moderately advanced, and far advanced stages, but also to the complications that occur in pulmonary disease. They have also employed the Weltmann reaction and compared it with the sedimentation rate in several examples of various types of tuberculosis over a prolonged period of time. The results are charted in the text. Although unable at the present to explain the mechanism of the Weltmann serum coagulation reaction, they believe that this test is of importance in the diagnosis and prognosis of disease, particularly in tuberculosis.

The technique follows:

From a stock solution of 10 per cent calcium chloride ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$), ten dilutions consisting of 0.1 per cent, 0.009 per cent, 0.08 per cent, 0.07 per cent, 0.06 per cent, 0.05 per cent, 0.04 per cent, 0.03 per cent, 0.02 per cent, and 0.01 per cent are made up. These dilutions are numbered from one to ten, beginning with the strongest concentration. Ten small test tubes, such as are used in the Wassermann reaction, are placed in a metal rack and numbered in order from 1 to 10. Into each tube is pipetted 5 c.c. of the similarly numbered calcium chloride solution and 0.1 c.c. of unhemolyzed serum. The tubes are then shaken so that the contents are well mixed and placed in a boiling water bath for fifteen minutes. They are then removed and the reaction can be immediately determined. The contents of the tubes may be clear, faintly opalescent, turbid, or there may be a flocculation. There is usually a sharp and easily noted difference between flocculation and turbidity.

The number of tubes in which coagulation occurs is designated by Weltmann as the coagulation band (C.B. of that particular serum). If there is a very slight or doubtful flocculation in one tube the reaction is interpreted as being intermediate between that tube and the one before it. In normal serum the first 6 tubes usually show flocculation. Sometimes there is a slight or doubtful flocculation in the seventh tube. The normal coagulation band is, therefore, 6 to $6\frac{1}{2}$, and is remarkably constant. This means that normal human serum diluted 50 times with calcium chloride solution and heated in a boiling water-bath for fifteen minutes will not coagulate or flocculate if the concentration of the calcium chloride is less than 0.04 per cent. If the coagulation band is less than 6, Weltmann speaks of the reaction as showing a shift to the left, whereas, if the coagulation band is 7 or greater, the reaction indicates a shift to the right.

NEPHROSIS Metabolic Study of Five Children With the Nephrotic Syndrome, Mitchell, A. G., Bittershofer, C. R., Wang, C. C., Kaucher, M., Wing, M., and Hogden, C. *Am J Dis Child* 55 27, 1938

Metabolic studies on five nephrotic children, two boys and three girls, between the ages of 3 and 16 years, were made during periods lasting from forty two to two hundred and fifty five days. In this report the clinical data, the effect of various treatments, and the records of excretion of urinary protein, studies of the blood basal metabolism, and the energy exchange are presented.

The effects of diuretics and dietary treatment varied greatly in producing temporary changes. However, no prolonged general improvement, including disappearance of edema, was accomplished with any procedure.

The protein content (albumin and globulin) of the serum, its nonprotein nitrogen content, and the cholesterol content of the blood were determined from time to time for the five patients. The values were those characteristic for the nephrotic syndrome, i. e., there were a low total protein content, a reversal of the ratio of albumin to globulin, a normal value for serum nonprotein nitrogen, except in two patients in whom the nephrotic syndrome was complicated, and a high content of blood cholesterol. The concentration of hemoglobin and the cell counts showed no definite changes.

The basal metabolism and the energy exchange are discussed in relation to age, height, and weight. The values are compared with data reported for normal children. The values for basal metabolism of all patients were within normal limits according to the standard of Benedict and Talbot and, with one exception, to the standard of Harris and Benedict. According to Dreyer's standard the heat production of all patients except one was low. From the determinations of caloric intake and caloric loss, calculations were made for the percentage absorption of energy and the expenditure of energy for growth, digestion, and activity. These values agreed with data obtained for normal children. This indicates that the energy exchange of a nephrotic patient is normal.

SULFANILAMIDE

Mall, J. W., and Smith, J. T. (*J A M A* 110 439 1938) report an actual lowering of the capacity of the blood for oxygen following the use of this drug.

Swift, H. F., Moen, J. K. and Hirst, G. K. (*J A M A* 110 426, 1938) conclude that the toxic action of sulfanilamide in active rheumatic fever so far outweighs the beneficial therapeutic effect that its use in this disease does not seem justified.

Bulger, J. A., Clifton, W. M., and Werner, M. (*J A M A* 110 343, 1938) report that sulfanilamide seemed to cause a depression of the white blood cells even to a point at which a leucopenia developed.

Agranulocytosis or granulopenia did not occur with this depression or leucopenia.

The action of sulfanilamide seems to be independent of the leucocytes in that it does not produce an increase in the total leucocytes or in the proportion of the polymorphous clear cells.

Cyanosis occurred in seven cases but spectroscopic examination of the blood specimens in these cases did not reveal bands of sulfhemoglobin or methemoglobin.

Sulfanilamide is a very effective drug in beta hemolytic streptococcus infections.

Frequent blood cell determinations should accompany sulfanilamide therapy.

Osgood (*J A M A* 110 349, 1938) discusses the mode of action and concludes that the major action of sulfanilamide on the beta hemolytic streptococcus seems to be neutralization of the toxins. Either because of this action or incidentally it also decreases the rate of cell division of this organism. It appears not to kill these organisms directly, al

though it does permit the bactericidal properties of human serum and to some extent phagocytosis by leucocytes to kill organisms which they otherwise would be unable to kill. It has no direct effect on phagocytosis.

The effective concentration of sulfanilamide would appear to be about 1:100,000 or only one tenth of that now ordinarily maintained in the blood stream, but this experimental observation requires confirmation by carefully controlled experiments on large numbers of human infections before it is justifiable to employ smaller dosages in dangerously ill patients. Sulfanilamide in concentrations even greater than those generally employed clinically does not appear to have direct toxic action on the nucleated cells of the majority of bloods or marrows. This does not exclude the occurrence of an occasional idiosyncrasy in the reactions of these cells such as is known to occur for other benzene ring drugs.

The possible effectiveness of and the mode of action of sulfanilamide on all other organisms known to produce human disease should be determined by the methods here described as soon as possible. Cultures of human marrow should aid materially in the study of the mode of action of both noxious and therapeutic agents.

Another case of fatal granulocytopenia is reported by Schwartz, W. F., Garvin, C. F., and Kaletsky, S. (J. A. M. A. 100: 368, 1938) and still another is recorded by Berg, S., and Holtzman, M. (J. A. M. A. 110: 370, 1938).

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr Warren T. Vaughan, Professional Building, Richmond, Va

The Control of Syphilis and Other Infectious Diseases

THIS volume presents the fifth series of Abraham Flexner lectures, presented in the School of Medicine of Vanderbilt University.

In view of the widespread publicity given to the campaign against syphilis and the frequent reference and the similar campaign conducted in Denmark, the first lecture "Control of Venereal Disease in Denmark With Special Reference to Syphilis," which, incidentally, was under Dr. Madsen's direction, will be read with great interest.

It is of particular interest to note in the account given in this lecture, that the attempt at control of syphilis through state agencies in Denmark began as long ago as 1774 and that the results widely quoted had their inception in a law promulgated in 1906.

The second lecture is concerned with the mechanism of bacterial infection, the third discusses tuberculosis in Denmark, the fourth, the influences of serons on infection, and the final lecture is devoted to whooping cough.

All these lectures are of scholarly quality and constitute real and valuable contributions to the subjects in question. This book may well be added to the physician's library.

The Diary of a Surgeon in the Years 1751-1752†

THIS is not a book to be started if there is an important appointment in the office, for once begun this book can only be laid down with reluctance.

There is some justification for sprinkling the proverbial grain of salt on publishers' announcements but that on the jacket of this book is not only true to the letter but, if anything, a little conservative.

It says, "This is one of those intriguing books which defies classification. It can be enjoyed as a vivid reconstruction of old medical practice, it can be read as a tale of high adventure on the sea, or it can be savored as a delightful piece of writing with a distinctive Pepysian flavor. Whatever it is, it makes thrilling reading—an unusual treasure of a book which does more to throw light on past days than reams of histories."

This is exactly that sort of a book. If you own it you won't want to lend it because, if you had borrowed it you would have to return it. If you do not read it you will miss a book undoubtedly destined to become a classic. If, for any reason, you have decided not to buy any more books for a while, do not open this one. You will not be able to resist it if you do.

For the benefit of the general reader a glossary of scientific names and a glossary of terms is included—wisely, for these rollicking pages will have a general and widespread appeal.

*Lectures on the Epidemiology and Control of Syphilis, Tuberculosis and Whooping Cough and Other Aspects of Infectious Disease. By Thorvald Madsen, M.D. Director of the State Serum Institute of Denmark, Copenhagen. Chairman of the Health Committee of the League of Nations. Cloth 216 pages 21 figures \$3.00. Williams & Wilkins Co. Baltimore, Md.

†The Diary of a Surgeon in the Year 1751-1752. By John Kniveton, Licentiate of the Society of Apothecaries, Doctor of Medicine of the University of Aberdeen, Teacher of Midwifery and Man-Midwife in Infirmary Hall, Surgeon's Mate H.M.S. Lancaster. Edited and Transcribed by Ernest Gray. Cloth 332 pages 7 illustrations \$2.50. D. Appleton Century Co. New York, N.Y.

Handbook of Medicine*

ANY book reaching a tenth edition must have proved itself useful and of value to those to whom it is addressed. This volume proves the rule. The present edition has been extensively revised and much new matter added throughout.

It may be predicted with confidence that this edition will meet the same cordial reception awarded to its predecessors.

The Hair and Scalp†

IN A REVIEW of the first edition of this book it was predicted that it would receive a cordial reception. The necessity for a second edition within two years is, hence, no surprise. This little volume will be found eminently practical, and a most useful addition to the practitioner's bookshelf. It can be recommended as an important contribution to a neglected subject.

The Medicolegal Aspects of the Ruxton Case‡

THIS is a volume of extraordinary interest. While Professor Glaister has achieved an international reputation as a medicolegal expert, it is doubtful whether his previous experience included a case with so many bizarre aspects, or presented so many difficulties as the case the story of which is here related.

The problem presented to the experts for the crown in the Ruxton case was the reconstruction and identification of two bodies, represented by over seventy gruesome fragments.

The bodies, those of Mrs. Ruxton and her maid-servant, had not only been dismembered but subjected to partial dissection and extraordinary mutilation in an effort to render identification impossible. To this task new methods were brought, in addition to those familiar to legal medicine, as a result of which identification was indubitably established, and the murderer, Dr. Ruxton, duly convicted and executed.

The book details through text and numerous illustrations the methods used. To the pathologist, the police surgeon, the lawyer, and all concerned with medicolegal problems this volume will prove a classic of its kind.

Vade Mecum of Medical Treatment§

THIS is a handbook the aim of which is to provide an account of the treatment of those conditions most commonly seen in general practice. To facilitate ready reference the diseases noted are arranged in alphabetical order. An index makes the contents of the book readily accessible.

The methods advocated throughout the book are either those with which the author has had personal experience, or those recommended by standard authorities.

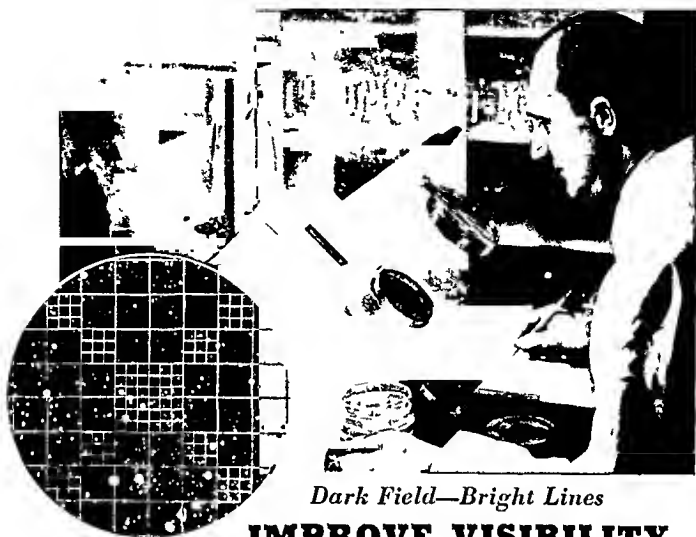
As a ready reference, this book should prove useful to the practitioner at large.

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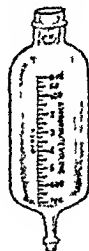
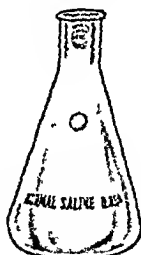
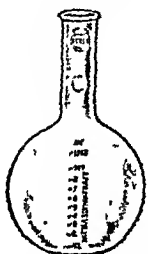
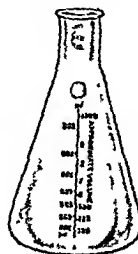
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